ABSCISIC ACID-REGULATED GROWTH MODULATIONS AND ITS APPLICATION FOR STRESS AND QUALITY MANAGEMENT OF VEGETABLE TRANSPLANTS

A Dissertation

by

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ABSTRACT

The goal of this study is to develop a management tool for producing high quality, more stress tolerant vegetable transplants and for prolonging transplant marketability. This study primarily involves physiological and morphological growth modulation by the stress hormone abscisic acid (ABA).

The first part of this study evaluated the effects of ABA foliar spray on stress and quality management of vegetable transplants. In muskmelon seedlings subjected to water withholding, pre-stress treatment of ABA improved the maintenance of leaf relative water content by limiting transpirational water loss. Upon re-watering, the ABA-treated seedlings showed faster photosynthetic recovery and greater dry matter accumulation than the untreated seedlings. In jalapeño pepper, ABA applied at the cotyledon to 3-leaf stage improved transplant compactness with minimal negative side effects. Although this method induced undesirable growth modifications in bell pepper and watermelon, ABA applied immediately before the transplant maturity stage was effective in delaying excessive shoot growth of bell pepper seedlings. These results demonstrate three beneficial effects of ABA for vegetable transplants: stress control, height control, and extension of transplant marketability.

The second part of this study examined the mechanisms of ABA-induced growth modulations in Arabidopsis: inhibition of leaf expansion, leaf chlorosis, and promotion of primary root elongation. Microscopic analysis of leaf epidermis revealed that ABA inhibits cell expansion, but not cell division or stomata formation, suggesting that the ABA-induced inhibition of leaf expansion is a mechanism to conserve water without limiting plant growth capacity. Leaf chlorosis induced by exogenous ABA occurred only in mature leaves and independently of ethylene synthesis. Tissue nitrogen (N) analysis with a ^{15}N-labeling technique

indicated a role of ABA as a regulator of N distribution. A proposed new mechanism is that ABA limits distribution of N into non-growing mature leaves, thereby inducing leaf-age dependent chlorosis. Using scanning electron microscopy (SEM), dehydration-induced root damage was characterized by thickening and deformation of root tips. Although exogenous ABA did not alleviate this damage, it promoted primary elongation especially under water stress. These results suggest that the overall function of ABA in stress adaptation is to conserve water and nutrients to support new growth.

DEDICATION

This dissertation is dedicated to my parents Ichiro and Terumi Agehara, my wife Yu-hsuan, my son Seishi, and my daughter Chiharu.

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NOMENCLATURE

A	Net CO_2 assimilation rate
ABA	Abscisic acid
C_i	Intercellular CO_2 concentration
DAM	Days after the anticipated maturity date
DAS	Days after sowing
DAT	Days after treatment
DBM	Days before the anticipated maturity date
DBT	Days before treatment
g_S	Stomatal conductance
FW	Fresh weight
NA	Numerical aperture
PEG	Polyethylene glycol
PPF	Photosynthetic photon flux
RWC	Relative water content
RSER	Relative stem elongation rate
SEM	Scanning electron microscopy
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Study Objectives and Approaches

The goal of this study was to develop a management tool for producing high quality, more stress tolerant vegetable transplants and for prolonging transplant marketability. This study primarily involved physiological and morphological growth modulation by the stress hormone abscisic acid.

The first part of this study (Chapter II) focused on developing ABA application strategies for stress and quality management of vegetable transplants. The test crops included muskmelon (*Cucumis melo* L.), bell and jalapeño pepper (*Capsicum annum* L.), and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai], all of which are major vegetable crops in Texas.

The second part of this study (Chapter III) used Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) as a model system and focused on understanding the mechanisms of ABA-regulated growth modulation.

1.2 Limitations of Vegetable Transplants

1.2.1 Transplant Shock

Vegetable seedlings often suffer transient water stress after transplanting. This so-called transplant shock is caused by the imbalance between water uptake and transpiration. In newly

transplanted seedlings, water uptake is reduced because of root injury during transplanting (Kramer, 1983) and disturbed root–soil contact (Burdett, 1990). In contrast to roots, shoots are relatively undamaged, maintaining high transpiration capacity. Moreover, upon transplanting, plants are exposed to direct sunlight, wind, and temperature extremes, which increase crop evapotranspiration. As a result, transpiration demands may exceed water uptake capacity by the limited root system, causing transient water deficits until normal growth can be reestablished. Therefore, minimizing post-planting water stress is essential for successful field establishment and subsequent crop production.

1.2.2 Excessive Stem Elongation

Vegetable transplants are typically produced in high-density plug trays at commercial nurseries (Marr and Jirak, 1990). For example, common seedling density for processing tomato (*Solanum lycopersicum* L.) is 2100 plants/m^2 with 288-cell plug trays (Garton 1990). Although increasing seedling density can reduce production costs per plant, it creates a competitive environment for light, which induces shade avoidance responses, such as internode and petiole elongation, inhibition of leaf expansion, and reductions in lamina thickness and specific stem weight. As a result, vegetable transplants grown at high-density are often characterized by tall and weak stems (Garner and Björkman, 1996; Smith, 1994). Such leggy transplants are considered unsuitable for shipping and transplanting, as they are susceptible to damage during these operations (Garner and Björkman, 1996; Shaw, 1993) and to wind lodging after transplanting (Garner and Björkman, 1999; Latimer and Mitchell, 1988). Consequently, their field establishment can be slow and non-uniform, potentially delaying early harvest and limiting marketable yield.

1.2.3 Limited Marketing Flexibility

Vegetable transplants quickly outgrow their marketability in commercial nurseries. Their limited marketing flexibility is a concern especially when transplanting is delayed because of inclement weather at the time of field establishment. Overmature transplants generally have spindly stems and excessive leaf growth, whereas their root growth is limited because of the small rooting volume of high-density plug trays (Marr and Jirak, 1990; Nishizawa and Saito, 1998). Such transplants are susceptible not only to damage during shipping and transplanting (Garner and Björkman, 1996; Shaw, 1993) but also to wind lodging after transplanting (Garner and Björkman, 1999; Latimer and Mitchell, 1988). In addition, the imbalance between transpiration demand and water uptake capacity can result in severe transplant shock and poor stand establishment (Agehara and Leskovar, 2012).

1.3 Abscisic Acid-regulated Growth Responses to Water Stress

1.3.1 Stomatal Closure

The mechanism of ABA-induced stomatal closure is hydroactive, which depends on metabolic processes in guard cells. First, ABA binds to ABA receptor proteins (Umezawa, 2011) and induces cytosolic Ca^{2+} elevations through extracellular influx and release from vacuoles (Schroeder et al., 2001). The elevated cytosolic Ca^{2+} level activates anion channels to promote anion release from guard cells, which in turn mediates the opening of outward K^+ channels (Schroeder et al., 2001). This net efflux of anions and K^+ is accompanied by osmotically mediated water movement out of the guard cells, leading to decreased guard cell turgor and stomatal closure. Stomatal closure is considered one of the first lines of defense against

immediate dehydration, because of its rapid response that effectively minimizes transpirational water loss (Chaves et al., 2002).

1.3.2 Inhibition of Leaf Expansion

Leaf growth inhibition can also occur in the absence of leaf turgor reductions during drought (Gowing et al., 1990; Passioura, 1988; Puliga et al., 1996), indicating regulatory processes that control leaf expansion rates in response to soil drying. One of the chemical signals proposed to be involved in this adaptive response is ABA. Accumulation of ABA occurs in leaves under water stress (Zeevaart and Boyer, 1984; Zeevaart and Creelman, 1988). Zhang and Davies (1990a; 1990b) reported that increasing ABA concentrations inhibited leaf expansion both *in vivo* and *in vitro*. Several studies suggest that restricted leaf expansion is correlated with ABA increases in xylem sap (Ismail et al., 2002; Salah and Tardieu, 1997) or leaves (Alves and Setter, 2000; He and Cramer, 1996; Van Volkenburgh and Davies, 1983).

Cellular responses to ABA may involve upregulation of potassium conductance and downregulation of proton efflux, which in turn inhibit cell expansion by membrane depolarization (Van Volkenburgh, 1999). In a study using ABA-deficit mutants, Bacon et al. (1998) demonstrated that ABA is required to mediate pH-regulated cell expansion in dehydrated barley (*Hordeum vulgare* L.). Whereas stomatal closure has an immediate effect in reducing water loss by transpiration, restricted leaf growth minimizes plant water use by limiting increases in transpirational capacity.

1.3.3 Maintenance of Root Elongation

In addition to leaves, ABA plays an important regulatory role in roots. Root growth is usually less inhibited than shoot growth under water deficit conditions (Creelman et al., 1990;

van der Weele et al., 2000; Watts et al., 1981). In maize (*Zea mays* L.) seedlings, Saab et al. (1990) proposed that endogenous ABA, which accumulates in root tips at low water potential, is required for the maintenance of primary root elongation. Their approach was to induce ABA deficiency by using fluridone, which limits ABA precursors by inhibiting carotenoid biosynthesis, or by using a mutant, in which carotenoid biosynthesis is deficient. Inhibition of ABA accumulation by either method resulted in severe reductions in root elongation at low water potential. This finding was confirmed in a subsequent study that showed a full recovery of root elongation when ABA in the elongation zone was restored to normal levels with exogenous ABA (Sharp, 1994). Furthermore, Sharp (2002) suggested that an important role of ABA in the maintenance of root elongation is to limit ethylene production.

In Arabidopsis, prolonged water stress induces development of short lateral roots, characterized by stubby tuberized structures (Vartanian et al., 1994). These specialized lateral roots enter a dormant mode and resume growth upon rehydration. This adaptive process is severely compromised in ABA-insensitive mutants such as *abi1-1*, suggesting that ABA is involved in the signaling of lateral root development.

1.3.4 Overall Effect

The overall effect of ABA is an increased biomass allocation in roots relative to shoots, which minimizes transpirational water loss, while maintaining high water uptake capacity. This morphological growth modulation and regulation of stomatal closure can collaboratively help plants cope with water stress (Taiz and Zeiger, 2010).

1.4 Abscisic Acid Function in Leaf Senescence

1.4.1 Leaf Chlorosis

Leaf chlorosis occurs as a result of chlorophyll breakdown during leaf senescence, and it is a negative quality characteristic for vegetable transplants. Several lines of evidence support the involvement of ABA in the regulation of leaf senescence. First, exogenous applications of ABA stimulate chlorophyll loss and leaf yellowing (Zacarias and Reid, 1990). Second, ABA accumulation coincides with a decline in chlorophyll content (Gepstein and Thimann, 1980). Third, ABA accumulation is suppressed when leaf senescence is delayed by exogenously applied kinetin (Gepstein and Thimann, 1980).

More recent studies provided insights into the genes and metabolic processes regulated by ABA during leaf senescence. The senescing effects of ABA are mediated by the expression of hydrolytic enzymes involved in chlorophyll breakdown (Weaver et al., 1998). Expression of several senescence associated genes (Weaver et al., 1998) and H_2O_2 accumulation (Hung and Kao, 2004) are also ABA-inducible and can promote leaf senescence. High availability of sugar can also trigger leaf senescence. However, in a study using Arabidopsis ABA deficient (*aba*) and insensitive (*abi*) mutants grown with varied glucose and N supply, Pourtau et al. (2004) demonstrated that ABA is not required for sugar-dependent regulation of leaf senescence. Instead, they suggested that, under water deficit conditions, maintenance of water relations by ABA may reduce stress-induced leaf chlorosis.

Ethylene is also known to play a role in leaf senescence, and ABA can promote leaf senescence through stimulation of ethylene production (Gepstein and Thimann, 1981). However, effects of ABA on leaf senescence are not fully mediated by ethylene (Zacarias and Reid, 1990). This ethylene-independent effect of ABA was demonstrated by Zacarias and Reid (1990), who

compared the leaf senescing effects of ABA and ethylene. When leaf discs of Arabidopsis wild type and ethylene insensitive mutant were treated with ethylene, chlorophyll loss was accelerated on the wild-type leaf discs, but no yellowing was observed on the leaf discs of ethylene insensitive mutant. By contrast, ABA treatment stimulated chlorosis in both wild-type and mutant leaf discs. Taken together, it is suggested that ABA acts as an initiating agent, whereas ethylene exerts its effect at a later stage of leaf senescence (Taiz and Zeiger, 2010).

1.4.2 Leaf Abscission

Leaf abscission is the final stage in leaf senescence, and it is an obvious quality defect for vegetable transplants. Ethylene is the principal promoter of the abscission process, whereas auxin acts as a suppressor of the ethylene effect (Morgan, 1984; Osborne, 1991). During leaf senescence, a reduction in the auxin gradient from leaf blade through the petiole increases ethylene production and ethylene sensitivity in the abscission zone (Brown, 1997). Ethylene induces the synthesis of hydrolytic enzymes, mainly cellulase (Mishra et al., 2008b; Tucker et al., 1991) and pectinase (Mishra et al., 2008a; Taylor et al., 1991), which in turn mediate degradation of the cell wall and middle lamella. Ethylene is an inhibitor of auxin biosynthesis, and increases in ethylene accelerate the abscission process (Ferrante and Francini, 2006). In contrast to ethylene, the role of ABA is generally recognized to be indirect, being mediated through stimulation of ethylene biosynthesis (Ferrante and Francini, 2006). In some cases, however, ABA plays an essential role in ethylene-induced leaf abscission. This was demonstrated by Suttle and Hultstrand (1993) using cotton seedlings, in which the accumulation of endogenous ABA was inhibited by norflurazon. Inhibition of ABA accumulation resulted in loss of ethylene-induced abscission, which was restored after treatment with exogenous ABA.

1.5 Application of Abscisic Acid for Stress and Quality Management of Vegetable Transplants

1.5.1 Water Stress Control

In terms of water stress, the main objective of ABA application in most previous studies has been to alleviate transplant shock by minimizing transpirational water loss during transplanting. Berkowitz and Rabin (1988) found that bell pepper seedlings dipped entirely in 1-mM ABA solution had higher stomatal resistance and leaf water potential than untreated seedlings after transplanting. When irrigation was withheld for 15 h after transplanting to impose water stress, the improved water status of the ABA-treated transplants resulted in increased field survival and yield. Similar results have been reported by Goreta et al. (2007). In their study, bell pepper seedlings were sprayed with ABA at 2000 $mg \cdot L^{-1}$ (7.6 mM) and subjected to water withholding in a greenhouse. They suggested that reductions in stomatal conductance by ABA enabled the maintenance of leaf water potential and prevented increases in electrolyte leakage and leaf abscission. On the other hand, Latimer (1992) reported that root-drench application of ABA at 660 $mg \cdot L^{-1}$ (2.5 mM) affected neither transplant growth nor field establishment of tomato seedlings under optimum irrigation. In maize seedlings, foliar application of 100-μM ABA increased root-to-shoot ratio but stimulated leaf chlorophyll degradations under water deficit conditions (Hejnák and Kykalová, 2009).

1.5.2 Height Control

Several gibberellin biosynthesis inhibitors, such as daminozide, paclobutrazol, and uniconazole, are commercially used in ornamental plant production to improve plant compactness, marketable value, and shelf life (Currey and Lopez, 2010). These chemicals are

highly active at low doses, long-lasting, and generally exhibit minimal undesirable consequences in many ornamental species (Blanchard and Runkle, 2007; Currey et al., 2012; Gibson and Whipker, 2001; Gibson and Whipker, 2003). However, their long-term growth inhibitory effects can be problematic for vegetable crops especially after transplanting (Cantliffe, 1993; Latimer, 1991). Furthermore, because of adverse health effects of these chemicals, uniconazole registered as Sumagic (Valent BioSciences, Libertyville, IL) is currently the only approved chemical for vegetable crops in the United States. According to its supplemental label, the approved vegetable crops include pepper, tomato, and eggplant (*Solanum melongena* L.).

In contrast to the gibberellin biosynthesis inhibitors, ABA can be rapidly inactivated by oxidation or conjugation (Davies and Jones, 1991). For example, leaf ABA can increase up to 50-fold within 4 to 8 h under water stress, but, upon re-watering, it declines to normal levels in the same amount of time (Zeevaart and Creelman, 1988). This rapid degradability suggests that ABA may be a suitable regulator for vegetable transplants, which require only transient growth suppression.

The potential of ABA as a height control agent has been studied mainly in bell pepper. For example, Leskovar and Cantliffe (1992) reported that the concentration effect of ABA on stem elongation was quadratic, with height suppression occurring above 10 μM. Biai et al. (2011) suggest that the effectiveness of height control is age-dependent, and that ABA application should be initiated at the cotyledon stage.

1.5.3 Extension of Transplant Marketability

Sumagic is registered primarily for height control and must be applied as a foliar spray during early development, no later than 14 d after two to four true leaf stage. This rather restrictive label limits its application as a plant growth retardant to prolong transplant

marketability. For vegetable transplants, a key characteristic of growth retardants is that they can be applied shortly before the anticipated transplant maturity stage to induce transient growth suppression to a predictable and manageable extent. Furthermore, this growth suppression must be followed by complete recovery, with no detrimental effects on transplant appearance or field performance.

The potential of ABA as a growth retardant has been studied for some vegetable transplants. Yamazaki et al. (1995) reported that cucumber (*Cucumis sativus* L.) and tomato seedlings sprayed with 0.38 or 1.89 mM ABA had reduced transpirational water loss and stem elongation during dark storage, thereby maintaining the overall quality and optimal size for transplanting. Sharma et al. (2006b) evaluated growth holding effects of ABA in tomato seedlings over 9 d after treatment. Although ABA had no significant height control effect, ABA at concentrations higher than 0.1 mM significantly reduced shoot fresh weight and total water use compared with control plants during the evaluation period.

CHAPTER II

STRESS AND QUALITY MANAGEMENT OF VEGETABLE TRANSPLANTS BY ABSCISIC ACID*

2.1 Study 1: Characterizing Concentration Effects of Exogenous Abscisic Acid on Gas Exchange, Water Relations, and Growth of Muskmelon Seedlings during Water Stress and Rehydration

Excess transpiration relative to water uptake often causes water stress in transplanted vegetable seedlings. Abscisic acid can limit transpirational water loss by inducing stomatal closure and inhibiting leaf expansion. The objective of this study is to examine the concentration effect of exogenous ABA on growth and physiology of muskmelon seedlings during water stress and rehydration. Plants were treated with seven concentrations of ABA (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) and subjected to 4-day water withholding. Application of ABA improved the maintenance of leaf water potential and relative water content, while reducing electrolyte leakage. These effects were linear or exponential to ABA concentration and maximized at 7.57 mM. Gas-exchange measurements provided evidence that such stress control is attributed to ABA-induced stomatal closure. First, net CO_2 assimilation rate (*A*) and stomatal conductance

* Parts of this chapter are reprinted with permission from "Characterizing concentration effects of exogenous abscisic acid on gas exchange, water relations, and growth of muskmelon seedlings during water stress and rehydration" by Agehara, S. and D.I. Leskovar, 2012. J. Amer. Soc. Hort. Sci., 137, 400-410 © (2012) American Society for Horticultural Science, "Growth reductions by exogenous abscisic acid limit the benefit of height control in diploid and triploid watermelon transplants" by Agehara, S. and D.I. Leskovar, 2014. HortScience, 49, 465-471 © (2014) American Society for Horticultural Science, and "Age-dependent effectiveness of exogenous abscisic acid in height control of bell pepper and jalapeño transplants" by Agehara, S. and D.I. Leskovar, 2014. Scientia Horticulturae, 175, 193-200 © (2014) Elsevier.

(g_s) Initially decreased with increasing ABA concentration by up to 95% and 70%, respectively. A follow-up study (≤ 1.89 mM ABA) confirmed this result with or without water stress and furtherrevealed a close positive correlation between A and intercellular CO_2 concentration 1 day after treatment ($r^2 > 0.83$). In contrast, ABA did not affect leaf elongation, indicating that stress alleviation was not mediated by leaf area adjustment. After 18 days of post-stress daily irrigation, dry matter accumulation showed a quadratic concentration-response, increasing with ABA concentration from 0 to 1.89 mM by 38% and 44% in shoot and roots, respectively, and decreasing at > 1.89 mM ABA by 16% to 18%. These results suggest that excess levels of ABA delay post-stress growth, despite the positive effect on the maintenance of water status and membrane integrity. Another negative side effect was chlorosis, which accelerated linearly with increasing ABA concentration, although it was reversible upon re-watering. The optimal application rate of ABA should minimize these negative effects, while keeping plant water stress to an acceptable level.

2.1.1 Introduction

Vegetable seedlings often suffer transient water stress after transplanting. This so-called transplant shock is caused by the imbalance between water uptake and transpiration. In newly transplanted seedlings, water uptake is reduced because of root injury during transplanting (Kramer, 1983) and disturbed root–soil contact (Burdett, 1990). In contrast to roots, shoots are relatively undamaged, maintaining high transpiration capacity. Moreover, upon transplanting, plants are exposed to direct sunlight, wind, and temperature extremes, which increase crop evapotranspiration. Successful field establishment depends on how quickly plants can recover water uptake capacity to support transpiration demand for normal growth.

Water stress increases accumulation of ABA in leaves (Davies and Jones, 1991). It is well documented that ABA acts as a stress signal which triggers adaptive changes in physiology and morphology of plants (Taiz and Zeiger, 2010). For example, ABA synthesized in roots or mesophyll is transported to guard cells where it promotes stomatal closure by inducing net efflux of potassium ions and thus reducing turgor pressure (Fan et al., 2004; Li et al., 2006; Schroeder et al., 2001). It is also known that ABA is involved in inhibition of leaf growth (Van Volkenburgh, 1999). Several studies reported that restricted leaf expansion was correlated with ABA increases in xylem sap (Ismail et al., 2002; Salah and Tardieu, 1997) or leaves (Alves and Setter, 2000; He and Cramer, 1996; Van Volkenburgh and Davies, 1983). In a study using ABA-deficient mutants, Bacon et al. (1998) demonstrated that ABA is required to mediate pH-regulated cell expansion in dehydrated barley. Whereas stomatal closure has an immediate effect in reducing transpirational water loss, restricted leaf expansion minimizes plant water use by limiting increases in transpirational area.

In addition to these functions in leaves, ABA plays an important regulatory role in root systems. Root growth is usually less inhibited than shoot growth under water deficit conditions (Creelman et al., 1990; Sharp et al., 2004; van der Weele et al., 2000; Watts et al., 1981). In maize seedlings, Saab et al. (1990) proposed that endogenous ABA, which accumulates in root tips at low water potential, is required for the maintenance of primary root elongation. Their approach was to inhibit ABA accumulation using fluridone, an inhibitor of the carotenoid (ABA precursor) biosynthesis pathway, or using a mutant with deficient carotenoid synthesis. Inhibition of ABA accumulation by either method resulted in severe reductions in root elongation at low water potential. This finding was further confirmed in a subsequent study that showed a full recovery of root elongation when ABA in the elongation zone was restored to normal levels with exogenous ABA (Sharp, 1994).

The overall effect of ABA can be summarized as an increase in root-to-shoot ratio, which, along with the regulation of stomatal closure, helps plants cope with water stress (Taiz and Zeiger, 2010). Thus, ABA application may reduce transplant shock in vegetable transplants. Berkowitz and Rabin (1988) found that bell pepper seedlings dipped entirely in 1 mM ABA solution had higher stomatal resistance and leaf water potential than untreated seedlings after transplanting. When irrigation was withheld for 15 h after transplanting to impose water stress, the improved water status by ABA resulted in increased field survival and yield. Similar results have been reported by Goreta et al. (2007). In their study, bell pepper seedlings were sprayed with ABA at 2000 $mg \cdot L^{-1}$ (7.6 mM) and subjected to two cycles of 4-d water withholding in a greenhouse. They suggested that reductions in g_s by ABA enabled the maintenance of leaf water potential and prevented increases in electrolyte leakage and leaf abscission. On the other hand, Latimer (1992) reported that root-drench application of ABA at 660 $mg \cdot L^{-1}$ (2.5 mM) affected neither transplant growth nor field establishment of tomato seedlings under optimal irrigation. In maize seedlings, foliar application of 100-μM ABA increased root-to-shoot ratio but stimulated leaf chlorophyll degradation under water deficit conditions (Hejnák and Kykalová, 2009).

The beneficial effects of exogenously applied ABA are not consistently evident in previous greenhouse and field studies. Most of these studies used a single concentration or narrow concentration range of ABA, which may not represent the optimal rate for the tested crop to promote desired responses. In fact, the magnitude of drought-induced increases in endogenous ABA varies among crop species, indicating a crop specific sensitivity to ABA (Davies and Jones, 1991). Furthermore, high-dose applications of ABA tend to have negative side effects, such as leaf chlorosis and abscission (Kim and van Iersel, 2011; Waterland et al., 2010c). Therefore, exogenous ABA must be tested over a wide range of concentrations to accurately evaluate its potential as a stress control agent. The objective of this study was to characterize

concentration effects of exogenous ABA on alleviating water stress and stimulating post-stress growth of muskmelon seedlings.

2.1.2 Materials and Methods

2.1.2.1 Abscisic Acid Solutions

The formulation of ABA used in this study was VBC-30025 (Valent BioSciences, Libertyville, IL) containing 90% of (+)-cis, trans-ABA. A stock solution was prepared according to the manufacture protocol using pre-weighed ABA and ethanol. Test solutions were prepared by diluting the stock solution with de-ionized water.

2.1.2.2 Plant Material

Muskmelon 'Caravelle' seeds were sown in a polystyrene tray with 128 inverted pyramid cells each containing 30 mL of peat-lite mix (Speedling Peat-lite; Speedling, Sun City, FL). Seedlings were grown at a commercial nursery greenhouse (Speedling) located in Alamo, TX for 40 to 45 d and then transferred to a greenhouse at Texas A&M AgriLife Research and Extension Center in Uvalde, TX (lat. 29°1'N, long. 99°5'W), where experiments were conducted in Oct. 2006 and May 2007. During seedling growth in the commercial nursery, average daily air temperature ranged from 17 to 30 °C and 6 to 27 °C in 2006 and 2007, respectively.

2.1.2.3 Growth Conditions and Treatments

In the first experiment (Study 1-1), 42-d-old seedlings were transplanted in a plastic tray (10.5 × 13 cm) with six cells each containing 60 mL of peat-lite mix. After transplanting, seedlings were fertilized with water-soluble fertilizer (20N–4.4P–16.6K) at 200 mg $N \cdot L^{-1}$ and watered daily thereafter. When seedlings were 45-d old, ABA solutions prepared at 0, 0.24, 0.47,

0.95, 1.89, 3.78, and 7.57 mM (0, 62.5, 125, 250, 500, 1000, and 2000 $mg \cdot L^{-1}$) were sprayed evenly over the seedlings using a hand-held sprayer between 1100 and 1200 HR. About 1 mL of ABA solution was applied per plant, which wetted the leaves thoroughly with little dripping. After spraying, seedlings were exposed to transient water stress by withholding water for 4 d. Irrigation was resumed when wilting occurred on all untreated (0 mM ABA) plants and performed daily thereafter. Seedlings were fertilized 11 d after ABA treatment (DAT) using the same rate as the first application and grown to 22 DAT. Day and night temperatures in the greenhouse were 20 to 32 °C and 15 to 22 °C, respectively, with a 11-h photoperiod. Maximum photosynthetic photon flux (*PPF*) at the canopy level was about 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

In the second experiment (Study 1-2), 51-d-old seedlings were transplanted individually in 10-cm square plastic pots (9.5 cm depth) containing 500 mL of peat-lite mix. After transplanting, seedlings were fertilized with water-soluble fertilizer (20N–4.4P–16.6K) at 200 $mg\ N \cdot L^{-1}$ and watered every 2 d thereafter. When seedlings were 55 d old, ABA solutions prepared at 0, 0.47, and 1.89 mM (0, 125, and 500 $mg \cdot L^{-1}$) were sprayed evenly over the seedlings using a CO_2-pressured backpack sprayer (model T; Bellspray, Opelousas, LA) between 1100 and 1200 HR. The CO_2 backpack sprayer was equipped with a six-nozzel hand-held boom and flat-fan nozzle tips (TP8002VS; TeeJet Technologies, Wheaton, IL) spaced 43 cm apart. Treatments were performed at 276 kPa to apply about 1 mL of ABA solution per plant, which wetted the leaves thoroughly with little dripping. Irrigated control plants were watered every 2 d throughout the experiment, whereas transiently dehydrated plants were subjected to 6-d water withholding and watered every 2 d thereafter. Irrigation was resumed when wilting was visible on the untreated (0 mM ABA) plants. Seedlings were fertilized at 12 DAT using the same rate as the first application and grown to 15 DAT. Day and night temperatures in the greenhouse were

25 to 32 °C and 15 to 26 °C, respectively, with a 14-h photoperiod. Maximum *PPF* at the canopy level was about 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

In both experiments, plants were watered between 0800 and 0900 HR by subirrigation until the growing medium was fully saturated. All fertilizer applications were performed by drenching into the growing medium through irrigation.

2.1.2.4 Gas Exchange

All gas-exchange measurements were made on an intact, unshaded, youngest expanded leaf, most often the third leaf from the apex, between 1200 and 1400 HR. Two leaves per replication, each from a different plant, were used.

In Study 1-1, A and g_s were measured using a closed-flow infrared gas analyzer (LI-6200; LI-COR, Lincoln, NE) at 0, 1, 2, 3, 4, 10, and 22 DAT. The instrument was equipped with a 0.25-L uncontrolled environment chamber which was customized to use a constant leaf area of 2.5 cm^2. Air flow rate was adjusted between 200 and 400 $\mu mol \cdot s^{-1}$ for each measurement to maintain constant relative humidity in the chamber. Ambient CO_2 concentration and canopy-level *PPF* during the measurements ranged from 390 to 410 $\mu mol \cdot mol^{-1}$ and 1000 to 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

In Study 1-2, the same variables as in Study 1-1 and intercellular CO_2 concentration (C_i) were measured using an open-flow infrared gas analyzer (LI-6400, LI-COR) at 0, 1, 2, 3, 5, 6, and 15 DAT. The instrument was equipped with a 2 × 3 cm leaf chamber and a red plus blue light-emitting diode light source (6400-02B, LI-COR). During measurements, photosynthetically active radiation, reference CO_2 concentration, air flow rate, and block temperature were maintained constant at 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 400 $\mu mol \cdot mol^{-1}$, 500 $\mu mol \cdot s^{-1}$, and 25 °C, respectively. Relative humidity in the sample chamber ranged between 50% and 70%.

2.1.2.5 Leaf Chlorophyll Index

Immediately after gas-exchange measurement, chlorophyll index was measured on the same leaves using a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Tokyo, Japan) in both experiments. Two readings were taken per leaf, about 1 cm from the leaf margin and between major leaf veins.

2.1.2.6 Plant Water Status (Study 1-1)

Water potential and relative water content (RWC) were measured on leaves of about the same age and size as those used for gas exchange measurements between 1200 and 1400 HR at 0, 2, 3, and 22 DAT. Leaf xylem pressure potential was measured as an estimate of leaf water potential using a pressure chamber (model 3005; Soil Moisture Equipment, Santa Barbara, CA) as described by Taiz and Zeiger (2010). Another set of leaves was sampled, and four 1-cm diameter discs were cut from each leaf with a cork borer avoiding major leaf veins. Fresh weight (FW) of two leaf discs was recorded to determine RWC. The other two were used to determine electrolyte leakage. The samples were floated on de-ionized water in a petri dish and hydrated in the dark. After 4 h, the turgid weight (TW) was recorded, and the samples were subsequently dried to a constant weight at 85 °C to determine the dry weight (DW). Relative water content expressed as a percentage was calculated as follows:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

2.1.2.7 Electrolyte Leakage (Study 1-1)

Electrolyte leakage was determined by a modified procedure of Blum and Ebercon (1981) to assess the degree of cell membrane damage. Two leaf discs per plant as described for RWC were rinsed with de-ionized water and placed in a capped 60-mL test tube filled with 10

mL of de-ionized water. The test tubes were incubated for 24 h at 25 °C on a rotary shaker set at 100 rpm, and electrical conductivity in the incubated solution (EC1) was measured using a conductivity electrode (sympHony SP40C; VWR International, Radnor, PA). The test tubes were then autoclaved for 15 min at 120 °C and 103 kPa, and electrical conductivity in the autoclaved solution (EC2) was measured upon equilibration at 25 °C. Electrolyte leakage expressed as a percentage was calculated as follows:

$$\text{Electrolyte leakage} = (EC1 / EC2) \times 100$$

2.1.2.8 Plant Growth (Study 1-1)

Leaves of about the same age and size as those used for other measurements were selected for leaf length measurements. Leaf length was measured from the lamina tip to the petiole attachment point non-destructively on the same leaves at 0 and 3 DAT. Relative leaf elongation rate (RLER) was calculated as follows:

$$RLER = d(\ln L) / dt$$

where $d(\ln L)$ is the difference in the natural logarithm of leaf length between two measurements and dt is the difference in time between two measurements.

At 22 DAT, two plants per replication were cut at the surface of growing medium and dried at 65 °C for 48 h to determine shoot dry weight. Roots were washed to remove the growth medium and dried at 65 °C for 48 h to determine root dry weight.

2.1.2.9 Statistical Design and Analysis

In Study 1-1, treatments were seven concentrations of exogenous ABA. There were three replicates (trays) and six subsamples (plants) for each treatment arranged in a randomized complete block design. Two plants per replication were used for each measurement. To

characterize the dose-responses of dependent variables to ABA, each data set was fitted to the following four models: linear Eq. [1], quadratic Eq. [2], exponential decay Eq. [3], and exponential rise to an asymptote Eq. [4].

$$Y = a + bx \quad [1]$$

$$y = a + bx + cx^2 \quad [2]$$

$$y = a + b\exp(-kx) \quad [3]$$

$$y = a + b[1 - \exp(-kx)] \quad [4]$$

where y is the predicted value of a dependent variable at ABA concentration x, and k is the rate constant. In Eqs. [1] and [2], a is the y intercept, b is the linear coefficient, and c is the quadratic coefficient. In Eq. [3], a is the lower asymptote, and b is the maximum decrease in y. The sum of a and b represents y for the control (0 mM ABA). In Eq. [4], a represents y for the control, and b is the maximum increase in y. The sum of a and b is the upper asymptote.

The model parameters were estimated using the NLMIXED procedure in SAS (version 9.2; SAS Institute, Cary, NC). The model with the smallest value of Akaike's information criterion was selected as the best model for each data set. Models were considered non-significant when the following model parameters were not significantly different from zero ($P > 0.05$): b in Eq. [1], c in Eq. 2, and b or k in Eqs. [3] and [4]. All models were fitted using all individual replicates (n = 3), although only mean values are shown in the figures below.

In Study 1-2, treatments were factorial combinations of two water stress levels (with or without water withholding) and three ABA concentrations. There were four replicates and two subsamples for each treatment arranged in a split plot design, with water stress as the main plot and ABA concentration as the subplot. All data analyses were, unless otherwise noted, run using the MIXED procedure with the Kenward–Rogers method (DDFM=KR) in SAS. When heteroscedasticity was indicated by a likelihood ratio test ($P \leq 0.05$), the MIXED procedure was

run with the GROUP option in the REPEATED statement. Main and interaction effects were tested using the restricted maximum likelihood method (METHOD=REML), in which water stress, ABA concentration, and the interaction were fixed factors, and replication and replication × water stress interaction were random factors. Multiple comparisons of least squares means were performed using the Tukey–Kramer test (ADJUST=TUKEY in the LSMEANS statement).

To assess stomatal and non-stomatal limitations to photosynthesis, a linear correlation between C_i and A was tested using the REG procedure in SAS. It was assumed that A is proportional to C_i under stomatal limitation (Lawlor, 2002). The r^2 values were calculated separately for the irrigated control and transiently dehydrated plants at each measurement time. The correlation was considered non-significant when the slope was not significantly different from zero ($P > 0.05$).

2.1.3 Results

2.1.3.1 Gas Exchange

Gas exchange showed a reversible inhibition in response to water stress and exogenous ABA in Study 1-1 (Figs. 2.1 and 2.2). Pre-treatment A (Fig. 2.1) and g_s (Fig. 2.2) were 10.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 1.2 mol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively. In the untreated control, A and g_s started to decrease at 2 and 1 DAT, respectively, and were reduced by more than 90% at 3 DAT. After re-watering, A recovered slowly to 21% of the pre-stress level, whereas g_s recovered to half the pre-stress level at 10 DAT and decreased thereafter. In most measurements, A and g_s showed similar concentration-dependent responses to ABA. At 1 DAT, A and g_s decreased with increasing ABA concentration by up to 95% and 70%, respectively. This dose-response was described by an exponential decay, with a steep decrease up to 0.95 and 3.78 mM ABA for A and g_s, respectively, followed by a gradual decrease. At 2 DAT, A and g_s continued to decrease with increasing ABA

concentration, but the slope became linear and less steep. Thereafter both A and g_s increased in response to ABA. Their dose-responses were quadratic at 3 DAT, with an increase up to 1.89 mM ABA followed by a decrease, and then they became a linear increase shortly after re-

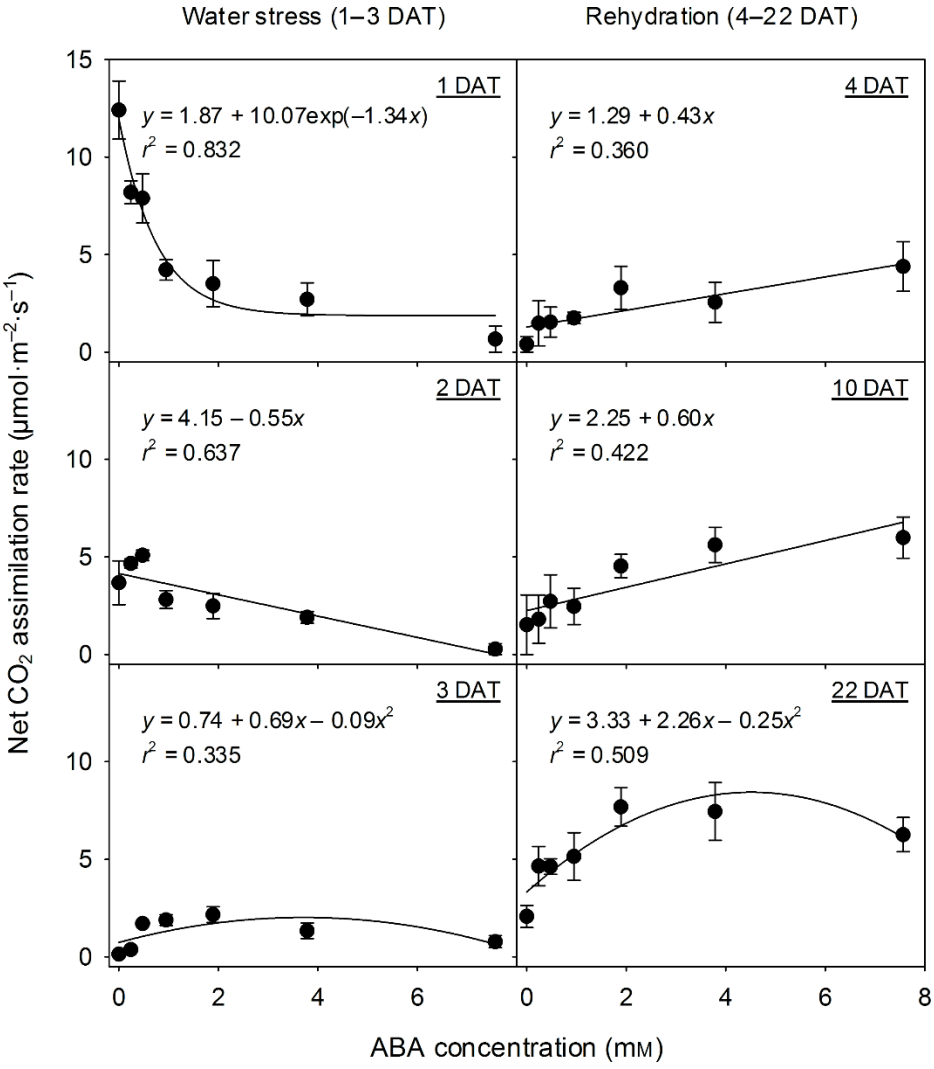

Fig. 2.1. Net CO_2 assimilation rate of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Study 1-1). Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 d after ABA treatment (DAT) and performed daily thereafter. Pre-treatment (0 DAT) mean ± SE was 10.4 ± 0.7 μmol·m^{-2}·s^{-1}. Data are means ± SE (n = 3). Solid lines show fits to the following models: exponential decay (1 DAT), linear (2, 4, and 10 DAT), and quadratic (3 and 22 DAT).

recovery to 73% of the pre-stress level at 1.89 mM, whereas that of g_s did not fit any tested regression models.

In Study 1-2, *A* was significantly affected by water stress, ABA concentration, or the interaction except at 2 DAT (Table 2.1). At 1 DAT, *A* decreased by nearly half with increasing

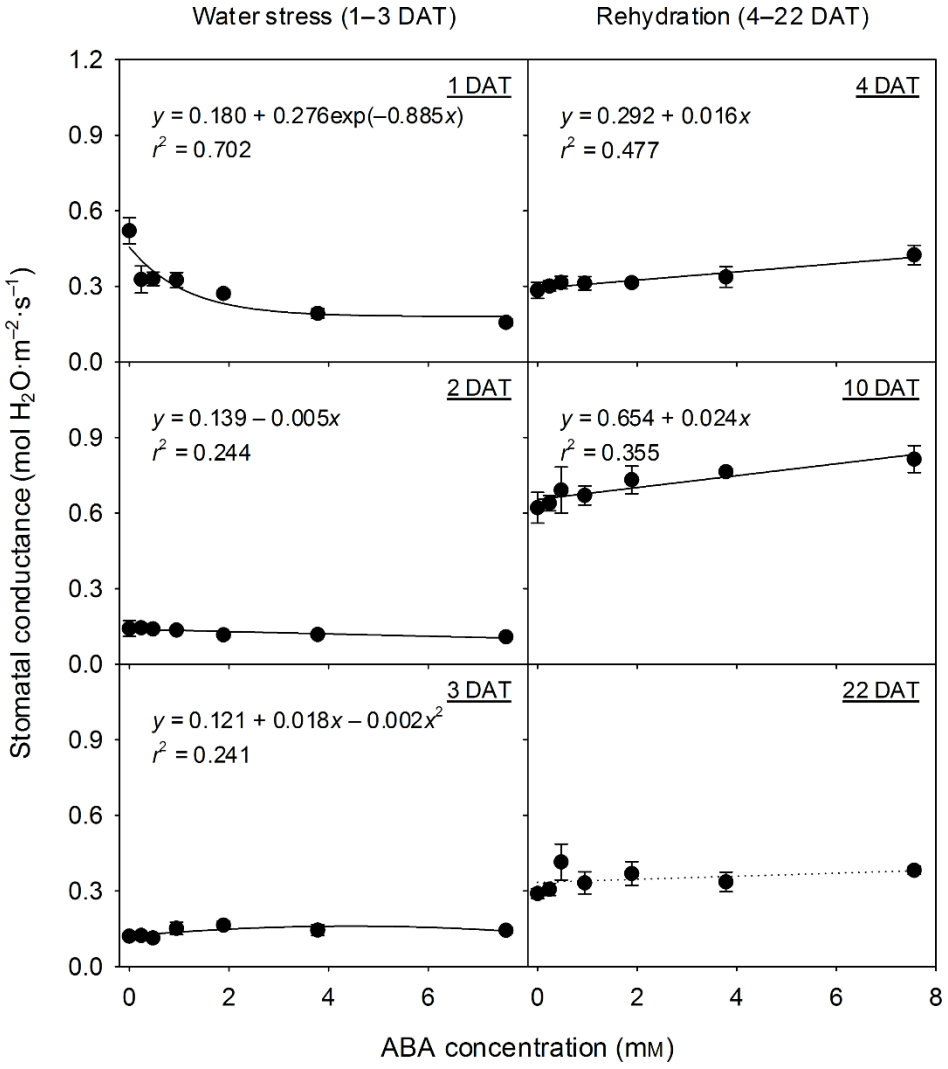

Fig. 2.2. Stomatal conductance of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Study 1-1). Treatments are as described in Fig. 2-1. Pre-treatment [0 d after treatment (DAT)] mean ± SE was 1.2 ± 0.1 mol $H_2O \cdot m^{-2} \cdot s^{-1}$. Data are means ± SE (n = 3). Solid lines show fits to the following models: exponential decay (1 DAT), linear (2, 4, and 10 DAT), and quadratic (3 DAT). A dotted line shows a non-significant ($P > 0.05$) linear trend.

Table 2.1. Effects of transient water stress and abscisic acid (ABA) spray concentration on net CO_2 assimilation rate of muskmelon seedlings (Study 1-2).

		Net CO_2 assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)						
	ABA concn[y]	Time after ABA treatment (d)						
Water stress[z]	(mM)	0	1	2	3	5	6	15
– Stress	0.00	16.9	16.8 ab[x]	16.4	16.3 ab	13.2 a	12.5 ab	10.8 ab
	0.47	15.9	12.8 abc	13.3	15.6 abc	12.8 a	9.6 abc	9.8 b
	1.89	17.0	8.5 c	12.8	18.5 a	14.5 a	11.3 abc	10.4 ab
			---------- Water withholding ----------				--- Rehydration ---	
+ Stress	0.00	17.5	17.1 a	12.4	10.8 c	5.9 b	6.9 c	10.1 ab
	0.47	17.4	11.2 bc	14.1	10.9 c	8.0 b	8.9 bc	11.8 ab
	1.89	16.6	9.4 bc	12.1	12.0 bc	12.2 a	12.9 a	12.9 a
Source of variation (*P* value)								
Water stress		0.879	0.941	0.203	0.000	0.010	0.213	0.034
ABA concn		0.947	0.000	0.277	0.171	0.000	0.006	0.188
Water stress × ABA concn		0.853	0.375	0.158	0.738	0.014	0.002	0.071

[z]Irrigated control plants (– Stress) were watered every 2 d throughout the experiment, whereas transiently dehydrated plants (+ Stress) were subjected to 6-d water withholding and watered every 2 d thereafter. Measurements at 6 d after ABA treatment were made 4 h after re-watering.
[y]Plants were sprayed with three concentrations of ABA solution (0, 0.47, and 1.89 mM) at 1 mL per plant.
[x]Mean separation in columns by the Tukey–Kramer test at $P \leq 0.05$.

ABA concentration. In the irrigated control (– Stress), *A* in the untreated (0 mM ABA) plants remained at the pre-stress level until 3 DAT, while that in the ABA-treated plants gradually recovered to the pre-stress level by 3 DAT. From 3 to 15 DAT, *A* steadily decreased by 34% to 44%, with no significant difference among the ABA treatments. Contrasting results were observed when water stress was imposed. In the untreated (0 mM ABA) plants, *A* decreased steadily from 1 to 5 DAT, being almost one-third of the pre-stress level at 5 DAT. The ABA-treated plants recovered *A* only from 1 to 2 DAT. From 2 to 5 DAT, *A* decreased to almost half the pre-stress level at 0.47 mM ABA, whereas it remained constant above two-thirds of the pre-stress level at 1.89 mM ABA. As a result, *A* showed a 2-fold increase with increasing ABA concentration at 5 DAT. This increase became gradually not significant after re-watering

because recovery of *A* was inversely proportional to ABA concentration. At 15 DAT, *A* averaged 11% higher in the stress treatment than the irrigated control ($P = 0.034$). In all measurements except 15 DAT, g_s responded to water stress and ABA similarly to, but to a greater extent than *A* (Table 2.2). In Study 1-2, C_i was also measured and regressed against *A* (Fig. 2.3). Regardless of water stress, they showed strong positive correlations ($r^2 > 0.83$) at 1 DAT, but r^2 values gradually declined thereafter. From 3 to 15 DAT, the correlations were weak and non-significant.

Table 2.2. Effects of transient water stress and abscisic acid (ABA) spray concentration on stomatal conductance of muskmelon seedlings (Study 1-2)[z].

		Stomatal conductance (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)						
	ABA concn	Time after ABA treatment (d)						
Water stress	(mM)	0	1	2	3	5	6	15
– Stress	0.00	0.363	0.462 ab[y]	0.315	0.374 ab	0.294 a	0.257 ab	0.701
	0.47	0.397	0.219 abc	0.233	0.414 a	0.259 ab	0.183 ab	0.636
	1.89	0.346	0.102 c	0.179	0.412 a	0.268 a	0.201 ab	0.606
			---------- Water withholding ----------				--- Rehydration ---	
+ Stress	0.00	0.338	0.468 a	0.190	0.158 b	0.071 c	0.125 b	0.471
	0.47	0.375	0.160 bc	0.227	0.149 b	0.094 bc	0.148 ab	0.627
	1.89	0.305	0.113 bc	0.144	0.168 b	0.171 abc	0.256 a	0.596
Source of variation (*P* value)								
Water stress		0.838	0.892	0.138	0.000	0.023	0.402	0.559
ABA concn		0.700	0.006	0.121	0.894	0.287	0.081	0.825
Water stress × ABA concn		0.989	0.895	0.379	0.896	0.119	0.011	0.279

[z]Treatments are as described in Table 2.1.
[y]Mean separation in columns by the Tukey–Kramer test at $P \leq 0.05$.

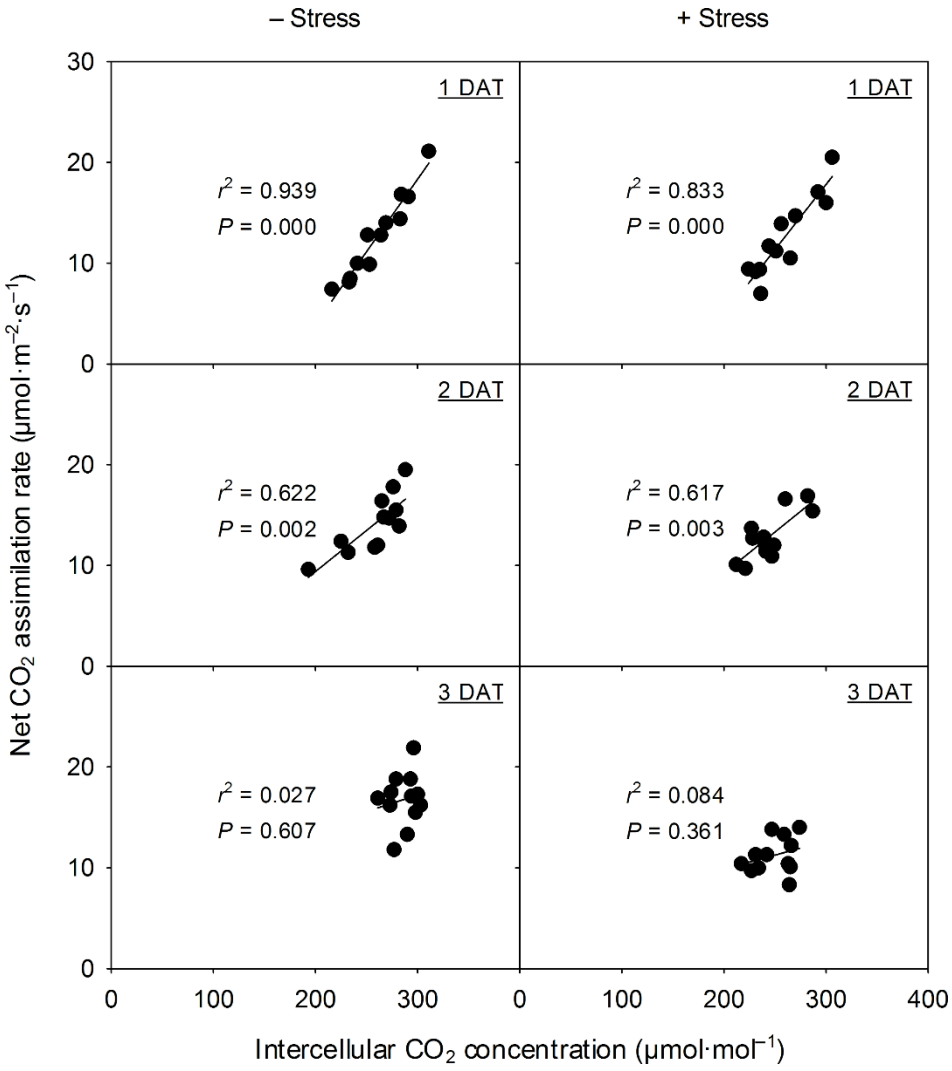

Fig. 2.3. Temporal declines in the linear correlation between intercellular CO_2 concentration and net CO_2 assimilation rate of muskmelon seedlings as affected by water stress (Study 1-2). Treatments are as described in Table 2.1. The correlation was weak ($r^2 < 0.2$) and non-significant ($P > 0.05$) from 3 to 15 d after ABA treatment (DAT). Data points are individual replicates (n = 4).

2.1.3.2 Leaf Chlorosis

Leaf chlorosis, as indicated by reductions in chlorophyll index, was induced by both water stress and exogenous ABA in Study 1-1 (Fig. 2.4). In the untreated control, chlorophyll index decreased only by 9% from 0 to 3 DAT (31.5 to 29.0) but decreased by more than half at 4 DAT (14.6), showing visible yellowing (Fig. 2.5). Thereafter chlorophyll index increased,

especially after fertilization at 11 DAT. Leaf chlorosis was mostly corrected by 22 DAT, with chlorophyll index recovering to 82% of the pre-stress level. In general, the dose-response of chlorophyll index to ABA was described as a linear decrease during the water stress period. The ABA-induced chlorosis progressed gradually, with the maximum chlorophyll loss by ABA

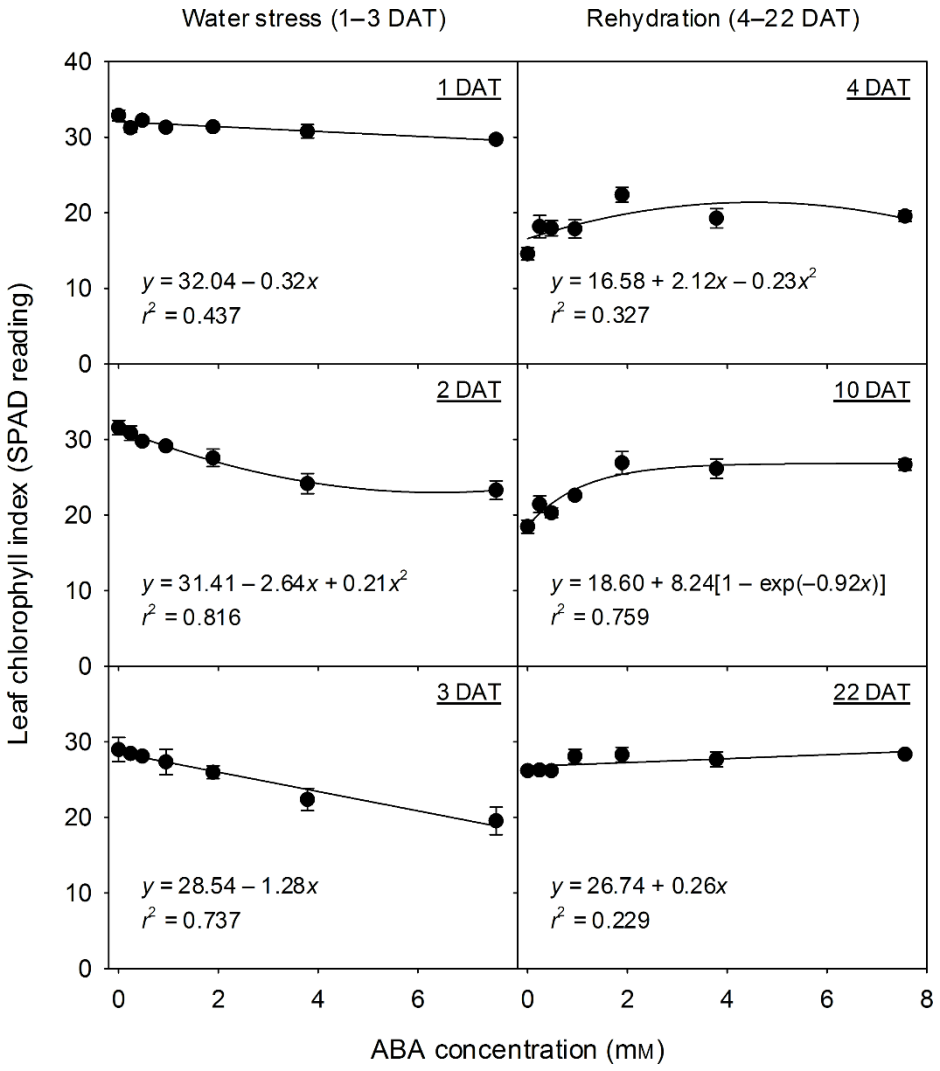

Fig. 2.4. Leaf chlorophyll index of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Study 1-1). Treatments are as described in Fig. 2-1. Pre-treatment [0 d after treatment (DAT)] mean ± SE was 31.5 ± 0.3. Data are means ± SE (n = 3). Solid lines show fits to the following models: linear (1, 3, and 22 DAT), quadratic (2 and 4 DAT), and exponential rise to an asymptote (10 DAT).

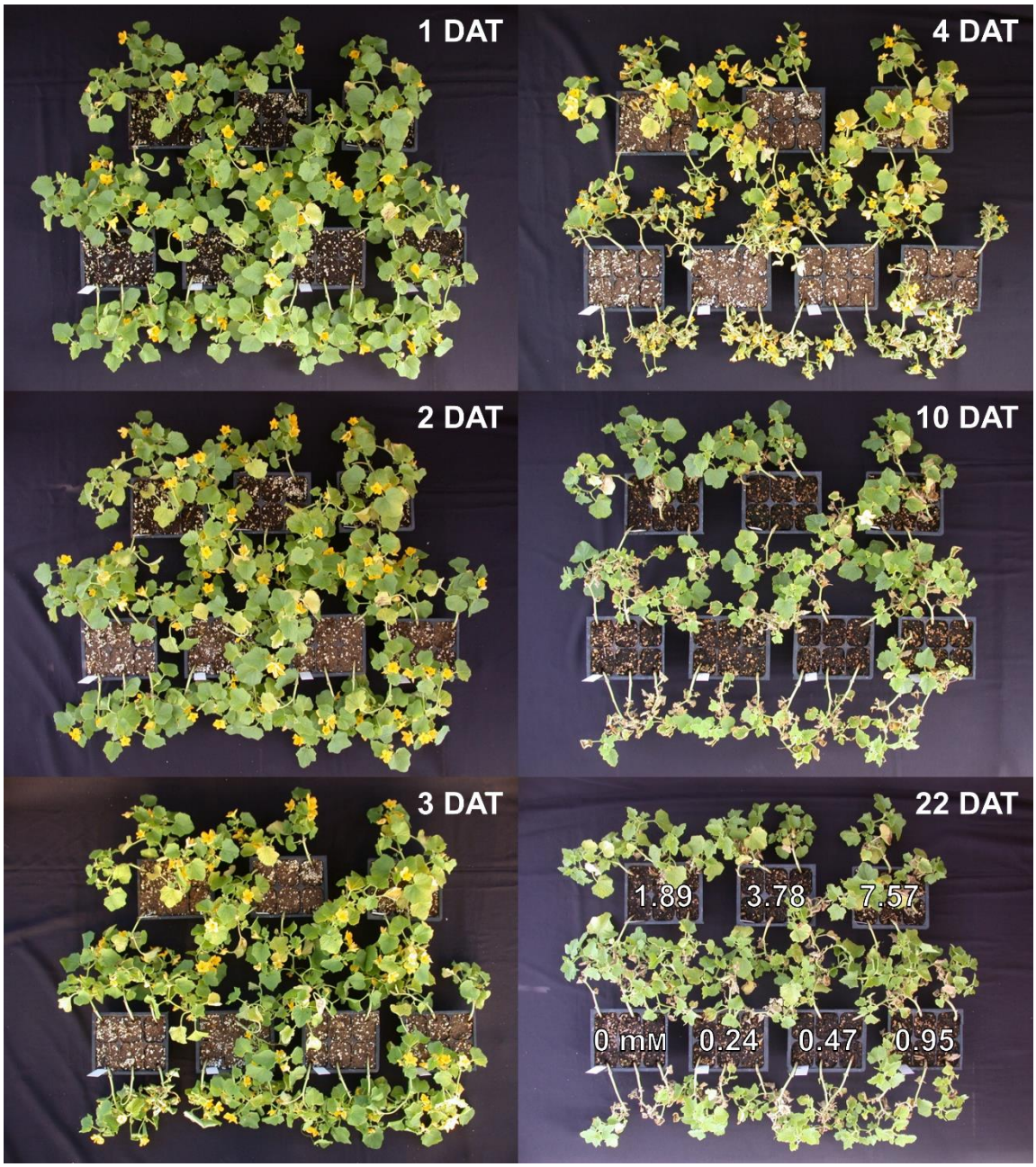

Fig. 2.5. Leaf chlorosis and wilting in muskmelon seedlings during water stress and rehydration as affected by abscisic acid (ABA) concentration (Study 1-1). Treatments are as described in Fig. 2-1 (from left to right and bottom to top in each image: 0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM). The image at 4 d after ABA treatment (DAT) was taken immediately before re-watering.

increasing from 10% at 1 DAT to 33% at 3 DAT. After re-watering, however, chlorophyll index increased in response to ABA. The dose-response was quadratic at 4 DAT, with an increase up to 1.89 mM ABA followed by a slight decrease, but, at 10 DAT, it became an exponential rise reaching a plateau at 1.89 mM ABA. The maximum increase in chlorophyll index by ABA was 54% and 46% at 4 and 10 DAT, respectively. At 22 DAT, the dose-response exhibited a very gradual linear increase.

Similar effects of exogenous ABA on chlorophyll index were observed in Study 1-2 (data not shown). Transient chlorosis was induced by exogenous ABA, regardless of water stress.

2.1.3.3 Plant Water Status

Leaf water potential averaged –0.12 MPa across all treatments at 0 DAT (Fig. 2.6A). In the untreated control, leaf water potential decreased from the pre-stress value by 5-fold (–0.62 MPa) at 2 DAT and by 14-fold (–1.76 MPa) at 3 DAT. The magnitude of these reductions was lowered exponentially with increasing ABA concentration; leaf water potential increased sharply up to 0.95 mM ABA and then increased gradually to a plateau. Relatively high leaf water potential (> –0.6 MPa) was maintained at ≥ 0.95 mM ABA throughout the water stress period. At 22 DAT, leaf water potential was similar to the pre-stress level in all treatments. Similar trends were observed for RWC (Fig. 2.6B). In the untreated control, RWC decreased from 93.6% at 0 DAT to 73.7% and 53.0% at 2 and 3 DAT, respectively. The magnitude of these reductions was lowered with increasing exogenous ABA in a linear manner. Thus, RWC was more indicative of mild stress than leaf water potential. Increasing ABA concentration maintained RWC as high as 92.7% and 89.8% at 2 and 3 DAT, respectively. Similarly to leaf water potential, RWC was restored by re-watering to the pre-stress level in all treatments.

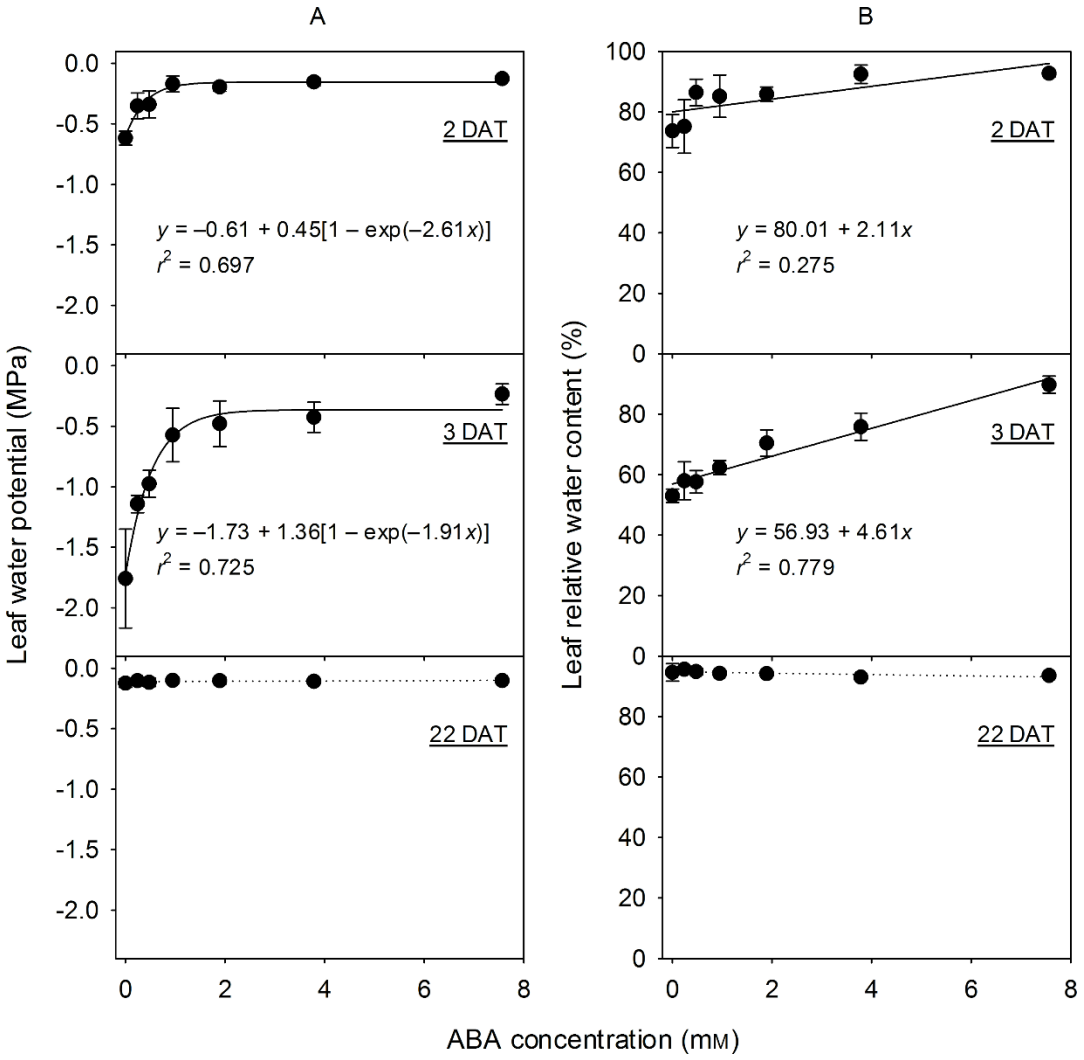

Fig. 2.6. Leaf water status of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Study 1-1): (**A**) water potential and (**B**) relative water content. Treatments are as described in Fig. 2-1. Pre-treatment [0 d after treatment (DAT)] means ± SE were –0.12 ± 0.01 MPa (**A**) and 93.6 ± 0.2% (**B**). Data are means ± SE (n = 3). Solid lines show fits to the following models: exponential rise to an asymptote (A) and linear (**B**). Dotted lines show non-significant ($P > 0.05$) linear trends.

2.1.3.4 Electrolyte Leakage

Electrolyte leakage averaged 36.1% at 0 DAT and remained nearly constant (35.1% to 41.0%) until 2 DAT in all treatments (Fig. 2.7). In the untreated control, electrolyte leakage

increased more than two-fold from 2 to 3 DAT (from 36.1% to 84.1%), indicating that cell membrane damage was caused by severe leaf dehydration. The magnitude of cell membrane damage decreased sharply with increasing ABA concentration up to 1.89 mM and then plateaued near the pre-stress level (38.2% to 41.0%). At 22 DAT, electrolyte leakage was reduced to the pre-stress level in all treatments.

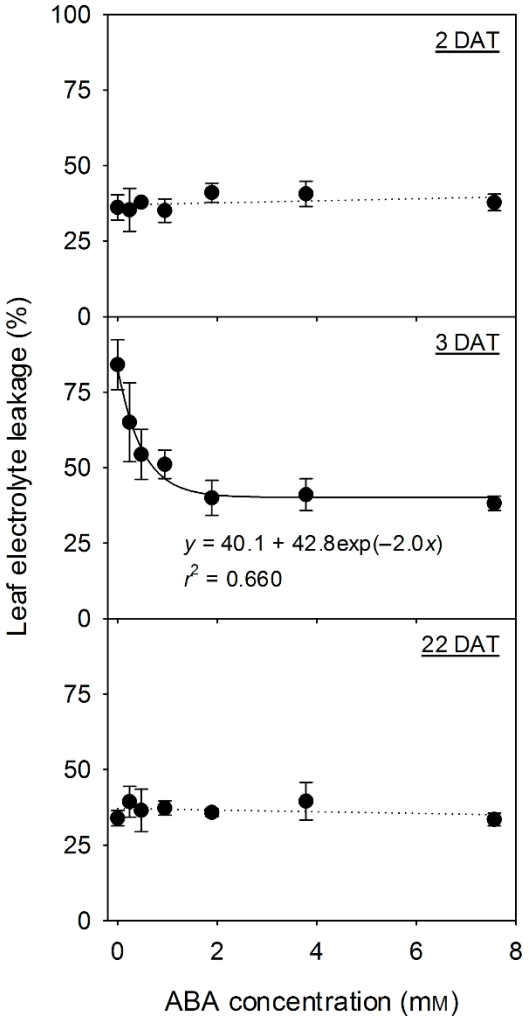

Fig. 2.7. Leaf electrolyte leakage of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Study 1-1). Treatments are as described in Fig. 2-1. Pre-treatment [0 d after treatment (DAT)] mean ± SE was 36.1 ± 4.0%. Data are means ± SE (n = 3). A solid line shows an exponential decay fit. Dotted lines show non-significant ($P > 0.05$) linear trends.

2.1.3.5 Plant Growth

Leaf length (Fig. 2.8A) and relative leaf elongation rate (Fig. 2.8B) were unaffected by exogenous ABA during the water stress period. At the end of the rehydration period, shoot dry weight showed a quadratic ABA dose-response, with an increase up to 1.89 mM ABA followed by a slight decrease (Fig. 2.9A). A similar but non-significant trend was found in root dry weight (Fig. 2.9B). The maximum dry matter increase by ABA application was 38% and 44% in shoot and roots, respectively. Root-to-shoot ratio ranged from 0.09 to 0.10 and did not fit any tested regression models (Fig. 2.9C).

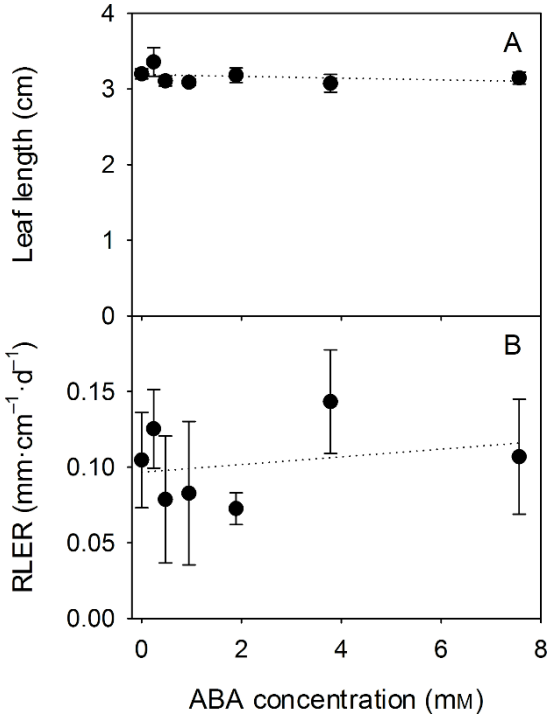

Fig. 2.8. Leaf elongation of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress (Study 1-1): (**A**) leaf length measured 3 d after ABA treatment (DAT) and (**B**) relative leaf elongation rate (RLER) between 0 and 3 DAT. Treatments are as described in Fig. 2-1. Data are means ± SE (n = 3). Dotted lines show non-significant ($P > 0.05$) linear trends.

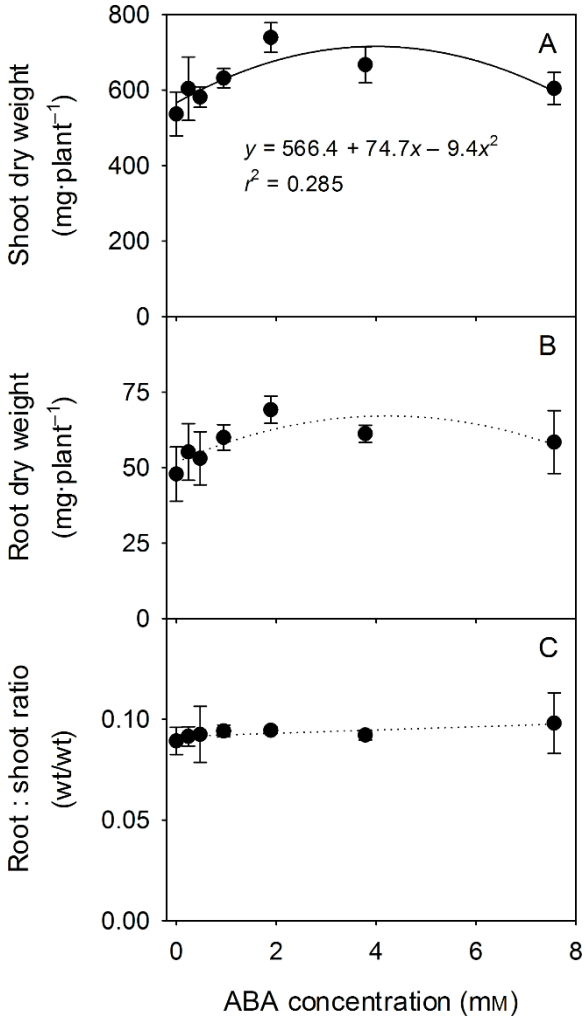

Fig. 2.9. Dry matter accumulation and partitioning of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration after recovery from water stress 22 d after ABA treatment (Study 1-1): (**A**) shoot dry weight, (**B**) root dry weight, and (**C**) root-to-shoot ratio. Treatments are as described in Fig. 2-1. Data are means ± SE (n = 3). A solid line shows a quadratic fit. Dotted lines show non-significant ($P > 0.05$) quadratic (**B**) and linear (**C**) trends.

2.1.4 Discussion

2.1.4.1 Abscisic Acid Reduces Water Stress by Promoting Stomatal Closure but Not by Inhibiting Leaf Growth

Foliar sprays of ABA applied before withholding water to muskmelon seedlings improved maintenance of leaf water potential and RWC (Fig. 2.6A and 2.B), thus minimizing

dehydration-induced damage to membranes (Fig. 2.7). These effects were linear or exponential to ABA concentration and were maximized at 7.57 mM. As a general rule, water stress can be classified as mild, moderate, and severe when RWC reductions are < 10%, 10% to 20%, and > 20%, respectively (Hsiao, 1973). According to these criteria, water stress at 2 DAT was moderate with ≤ 0.24 mM ABA and mild with ≥ 0.47 mM ABA, while water stress at 3 DAT was classified as severe with ≤ 1.89 mM ABA, moderate with 3.78 mM ABA, and mild with 7.57 mM ABA. Such a stress gradient also was evident by the severity of wilting (Fig. 2.5).

The alleviation of water stress may be associated with ABA-induced acclimation to water limiting conditions. Stomatal closure is considered one of the first lines of defense against immediate dehydration (Chaves et al., 2002), and its regulation is known to be mediated by ABA. In this study, A and g_s initially decreased with increasing ABA concentration by up to 95% (Fig. 2.1) and 78% (Table 2.2), respectively, suggesting that ABA-induced stomatal closure allowed rapid and dramatic water conservation at the expense of CO_2 supply to photosynthesis.

In addition to stomatal closure, ABA is involved in the inhibition of leaf expansion (Alves and Setter, 2000; Bacon et al., 1998; He and Cramer, 1996), which can reduce plant water use by limiting increases in transpirational area. However, leaf elongation during the water stress period was unaffected by exogenous ABA (Fig. 2.8). Because leaf length is an accurate indicator of leaf area in muskmelon (Panta and NeSmith, 1995), this result suggests that ABA-induced acclimation to water stress may not be mediated by leaf area adjustment in muskmelon seedlings. Cell expansion is a turgor-driven process and is extremely sensitive to dehydration (Taiz and Zeiger, 2010). Since plant water loss was inversely proportional to ABA concentration (Fig. 2.6A and B), turgor reduction may have limited cell expansion more severely at lower ABA concentrations, thereby potentially masking the effect of ABA on leaf expansion. However, in a previous study using pepper seedlings subjected to two cycles of 4-d water

withholding, the maintenance of plant water status by exogenous ABA was associated with reductions in both g_s and leaf area (Goreta et al., 2007). These contrasting results indicate that ABA may regulate differential acclimation strategies depending on plant species.

2.1.4.2 Dose-response of Gas Exchange to Abscisic Acid during Water Stress and Recovery

The initial dose-response of gas exchange to ABA was described by an exponential decay, with a steep decrease up to 0.95 and 3.78 mM ABA for A and g_s, respectively, followed by a gradual decrease (Figs. 2.1 and 2.2). This change in slope gradient suggests that gas exchange became less responsive to increases in ABA above those high concentrations. The mechanism of ABA-induced stomatal closure is hydroactive, which depends on metabolic processes in guard cells. First, ABA binds to ABA receptor proteins (Umezawa, 2011) and induces cytosolic Ca^{2+} elevations through extracellular infux and release from vacuoles (Schroeder et al., 2001). The elevated cytosolic Ca^{2+} level activates anion channels to promote anion release from guard cells, which in turn mediates the opening of outward K^+ channels (Schroeder et al., 2001). This net efflux of anions and K^+ is accompanied by osmotically mediated water movement out of the guard cells, leading to decreased guard cell turgor and stomatal closure. Therefore, ABA receptors, ion channels, and ions themselves (e.g., Ca^{2+}, Cl^-, K^+, and organic anions) may be limiting factors for ABA signal transduction and thus effectiveness of exogenous ABA in inducing stomatal closure.

Under prolonged water stress, the dose-response of gas exchange shifted to a quadratic function, with A and g_s increasing up to 1.89 mM ABA and decreasing with higher ABA concentrations (Figs. 2.1 and 2.2). As noted above, water loss and cell membrane damage progressed more severely at lower ABA concentrations (Figs. 2.6 and 2.7). Such impaired water relations can strongly inhibit enzymatic activities and cellular metabolism involved in

photosynthetic processes (Lawlor, 2002). Moreover, rapid dehydration of leaf tissue can cause hydropassive stomatal closure independently of ABA. That is, when evaporative water loss from guard cells is faster than water movement from adjacent epidermal cells, guard cell turgor decreases, forcing stomata to close (Taiz and Zeiger, 2010). Similarly, stomata will remain closed if plant tissue is too dehydrated to permit guard cell turgor. Therefore, the quadratic response of A and g_s can be explained that metabolic impairment and hydropassive stomatal closure were the major limiting factors below 1.89 mM ABA, while above this concentration hydroactive stomatal closure by exogenous ABA was more inhibiting to gas exchange.

After re-watering, gas exchange generally increased linearly with increasing ABA concentration (Figs. 2.1 and 2.2). It is likely that ABA permitted maintenance of tissue hydration and membrane integrity (Figs. 2.6 and 2.7) and minimized metabolic impairment (Lawlor, 2002), thereby enabling a fast recovery of gas exchange with rehydration. Additionally, inhibition of gas exchange by exogenous ABA is reversible by re-watering with no negative impact on subsequent recovery. This conclusion was also confirmed under well-watered conditions, where complete recovery of gas exchange occurred within 3 d of ABA treatment (Tables 2.1 and 2.2). The transient effect of ABA is probably due to oxidation or conjugation that rapidly inactivates ABA in plant tissue (Davies and Jones, 1991). In contrast, ABA analogs (synthetic chemical structures) are known to have long-term consequences because of their high chemical stability (Abrams et al., 1997). Thus, to control short-term water stress (e.g., transplant shock, but not prolonged drought), the easily degradable natural ABA may be more suitable than its analogs.

2.1.4.3 Stomatal and Non-stomatal Limitations to Photosynthesis

Although stomatal closure is an efficient strategy to conserve water, restricted entry of CO_2 lowers C_i and consequently limits A (Lawlor, 2002). This stomatal limitation to photosynthesis was demonstrated by high r^2 values (> 0.83) for the positive correlation between C_i and A at 1 DAT (Fig. 2.3). In the absence of water stress, the subsequent decline in r^2 value was due to the recovery in both C_i and A, indicating stomatal re-opening resulting from degradation of exogenous ABA. In contrast, during water withholding the decline in r^2 value occurred without the recovery in C_i and A, suggesting that non-stomatal factors became more important with progressive water stress. Non-stomatal limitations may have been associated with impaired enzymatic activities and cellular metabolism, which are known to inhibit photosynthetic processes independently of CO_2 supply (Lawlor, 2002).

2.1.4.4 Abscisic Acid Induces Transient Chlorosis but Reduces Water Stress-induced Chlorosis

Leaf chlorosis is reported as a negative side effect of exogenous ABA in various crops (Blanchard et al., 2007; Hejnák and Kykalová, 2009; van Iersel et al., 2009; Waterland et al., 2010a). In this study, gradual but only transient leaf chlorosis was induced by exogenous ABA in muskmelon seedlings (Fig. 2.4). Notably, this chlorosis was accelerated linearly with increasing ABA concentration. Severe symptoms displayed uniform chlorosis across the entire lamina (Fig. 2.5) with up to 33% of chlorophyll loss by ABA (Fig. 2.4). The magnitude of chlorosis was comparable to that reported by Waterland et al. (2010c), who found 25% to 85% of chlorophyll loss in pansy (*Viola* × *wittrockiana* Gams.) and viola (*Viola cornuta* L.) drenched with 0.95 mM ABA or sprayed with 1.89 mM ABA. The ABA-induced chlorosis can be attributed to the senescing effects of ABA, resulting from the gene expression of hydrolytic enzymes involved in chlorophyll breakdown (Weaver et al., 1998) or the stimulation of ethylene

production (Gepstein and Thimann, 1981). A lack of nutrients, particularly N and Mg, is another factor promoting leaf chlorosis (Marschner, 1995). Since their uptake depends mainly on transpiration-driven mass flow (Havlin et al., 1999), reduced transpiration caused by ABA application may have limited N and Mg supply for chlorophyll formation and thus contributed to leaf chlorosis.

Leaf chlorosis was induced also by water stress mainly from 3 to 4 DAT, during which chlorophyll degradation was inversely proportional to ABA concentration (Fig. 2.4). As a result, chlorosis became most severe in the untreated control, while it was minimized at ≥ 1.89 mM ABA. It seems that water stress induces chlorosis only during the severe stress period. It is also important to note that this process can develop very rapidly once initiated. The alleviation of chlorosis by ABA application was thus likely derived from maintenance of plant water status that minimized chlorophyll loss by dehydration. This effect remained significant during the post-stress recovery of chlorophyll. These results suggest not only that ABA-induced chlorosis is reversible upon re-watering, but also that ABA can reduce leaf chlorosis depending on the degree of water stress.

2.1.4.5 Effects of Abscisic Acid on Post-stress Growth

Although tissue dehydration and electrolyte leakage were minimized at the highest ABA concentration (Figs. 2.6 and 2.7), post-stress dry matter accumulation increased only up to 1.89 mM and decreased slightly with higher ABA concentrations (Fig. 2.9A and B). This observation raises the question of how excess levels of ABA negatively affected post-stress growth, despite the positive effect on the maintenance of water status and membrane integrity. One explanation could be the excessive inhibition of photosynthesis by ABA before re-watering, which may have limited the supply of assimilates for dry matter production (Amthor, 2007).

Under water deficit conditions, ABA accumulation in leaves can suppress shoot growth (Taiz and Zeiger, 2010), while that in root tips is required for the maintenance of primary root elongation (Sharp et al., 2004; Spollen et al., 2000). However, exogenous ABA did not affect root dry matter partitioning in muskmelon seedlings (Fig. 2.9C). The lack of preferential root growth in response to ABA application may be due to the small rooting volume in our trays that restricted the capacity for root elongation (Nishizawa and Saito, 1998).

The effectiveness of ABA application in promoting post-stress growth appears to be determined by the balance between water stress control and inhibition of photosynthesis. Therefore, the expected degree of water stress and sensitivity of the targeted crop to exogenous ABA must be considered to determine the optimal application rate.

2.2 Study 2: Age-dependent Effectiveness of Exogenous Abscisic Acid in Height Control of Bell Pepper and Jalapeño Transplants

Height control of vegetable transplants is important for improving their adaptability to shipping and transplanting operations. Abscisic acid inhibits stem elongation but can also induce undesirable growth modification. To optimize its application timing for effective height control, age-dependent sensitivity of various growth variables to ABA was examined in two pepper cultivars. Bell pepper 'Excursion II' seedlings were sprayed once with 3.8 mM ABA at 25, 18, or 11 days before transplanting (DBT), or twice with 1.9 mM ABA at 25 and 18 DBT. Jalapeño 'Colima' seedlings were sprayed once with 3.8 mM ABA at 22, 15, or 8 DBT, or twice with 1.9 mM ABA at 22 and 15 DBT. For all treatments, the application rate was 0.71 mg ABA per plant with the spray volume of 0.61 L m^{-2} (0.71 ml/plant). Only 'Excursion II' maintained significantly shorter plant height in all ABA treatments until the transplanting stage, ranging

from 80% to 88% of the control. By contrast, leaf chlorosis and overall growth delay were induced by ABA in 'Colima'. Age-dependent sensitivity to ABA was evident in leaf area of both cultivars, and in stem diameter and shoot and root biomass of jalapeño 'Colima', all of which showed maximal reductions when 3.8 mM ABA was applied at the cotyledon stage (first application). These results suggest that ABA is effective in height control for bell pepper 'Excursion II', and that it should be applied at least one week after the emergence of first true leaf to minimize the negative side effects. Importantly, subsequent field evaluations demonstrated that the growth modulation by ABA was only transient with no negative impact on marketable yield.

2.2.1 Introduction

Height control of vegetable transplants is important for maintaining quality during shipping and improving adaptability to transplanting operations (Björkman, 1999; Latimer, 1998). Because vegetable transplants are typically grown in high-density plug trays (Marr and Jirak, 1990), stems can grow excessively elongated and weak as a result of shade avoidance responses (Smith, 1994). Compared with stocky transplants, such weak transplants are more difficult to handle and are easily damaged during shipping (Garner and Björkman, 1996). They are also more susceptible to damage during mechanical transplanting (Shaw, 1993) and to lodging in the field (Garner and Björkman, 1999; Latimer and Mitchell, 1988). As a result, their field establishment can be slow and non-uniform, delaying growth and early harvest and potentially limiting marketable yield.

The cellular basis for stem elongation is a combination of cell division and cell elongation, both of which are stimulated by gibberellins (Sachs, 1965; Taiz and Zeiger, 2010). Ethylene has antagonistic effects by inhibiting cell elongation and promoting stem thickening

(Zarembinski and Theologis, 1994). In ornamentals and flowers, several gibberellin inhibitors, such as daminozide, paclobutrazol, and uniconazole, are commercially used to produce compact plants (Gibson and Whipker, 2001; Whipker et al., 2000). However, as they have long-term growth inhibiting effects (Cantliffe, 1993; Latimer, 1991), only uniconazole is currently available for vegetable crops at relatively low application rates to avoid phytotoxicity. Alternatively, stem elongation can be reduced by mechanical stimulation that increases ethylene production (Baden and Latimer, 1992; Björkman, 1999; Garner and Björkman, 1997; Hiraki and Ota, 1975). Such mechanical treatments include brushing the upper canopy, shaking, and vibration by wind or forced aeration, but their commercial application is limited by high costs of automation and labor (Latimer, 1998).

Abscisic acid is another plant growth regulator, which exogenous application inhibits stem elongation (Latimer and Mitchell, 1988; Leskovar and Cantliffe, 1992; Yamazaki et al., 1995). In contrast to gibberellin inhibitors, ABA can be rapidly inactivated in plant tissues by oxidation or conjugation (Davies and Jones, 1991), suggesting that it may be more suitable for vegetable transplants that require only transient growth inhibition. The potential of ABA to control transplant height has been studied in bell pepper. For example, Leskovar and Cantliffe (1992) reported that the concentration effect of ABA on stem elongation was quadratic, with height suppression occurring above 10 μM. Biai et al. (2011) suggest that the effectiveness of height control is age-dependent, and that ABA application should be initiated at the cotyledon stage. However, this recommendation is based solely on plant height, although other growth components are also known to be affected by ABA (Taiz and Zeiger, 2010). Moreover, high-dose applications of ABA tend to have negative side effects, such as leaf chlorosis and abscission (Agehara and Leskovar, 2012; Kim and van Iersel, 2011; Waterland et al., 2010c).

Therefore, understanding overall growth modification will provide the basis to further optimize ABA application methods for height control.

Our first objective was to examine the age-dependent sensitivity of various growth variables to ABA in bell pepper and jalapeño seedlings. Such information will be useful to determine the application timing for the most effective height control. To justify the advantages of height control, our second objective was to evaluate field performance of the ABA-treated transplants.

2.2.2 Materials and Methods

2.2.2.1 Plant Material and Growth Conditions

Seeds of two pepper cultivars (Abbott & Cobb, Feasterville, PA), bell pepper 'Excursion II' and jalapeño 'Colima', were sown on 16 Feb. and 6 Mar. 2010, respectively, in a polystyrene tray with 200 inverted pyramid cells each containing 23 mL of peat-lite mix (Speedling Peat-lite; Speedling). Seedlings were grown at a commercial nursery greenhouse (Speedling) located in Alamo, TX until they reached optimal size for transplanting according to the nursery's commercial standard. Average daily air temperature during seedling growth ranged from 9 to 27 °C.

2.2.2.2 Abscisic Acid Treatments

There were five treatments for each cultivar: no spray control, three timings of a single spray with 3.8 mM (1000 mg L^{-1}) ABA, and one treatment of a double spray with 1.9 mM (500 mg L^{-1}) ABA. The single spray was performed at 25, 18, and 11 DBT [17, 24, and 31 d after sowing (DAS)] for 'Excursion II' and at 22, 15, and 8 DBT (19, 26, and 33 DAS) for 'Colima'. The double spray was performed at 25 and 18 DBT for 'Excursion II' and at 22 and 15 DBT for

'Colima'. Seedlings had fully opened cotyledons with one or two immature true leaves at the time of the first ABA application. Spray volume was set at 0.61 L m^{-2} (0.71 ml/plant), which wetted the leaves thoroughly to the dripping point. The resulting application rate was 0.71 mg ABA per plant in all ABA treatments.

The formulation of ABA stock solution was VBC-30151 containing 10% of S-ABA, a naturally occurring active form in plants. Test solutions were prepared immediately before each treatment by diluting the stock solution with irrigation water at the nursery. CapSil (Aquatrols, Paulsboro, NJ) was added at 0.05% (v/v) as an adjuvant according to the manufacture's protocol (Valent BioSciences), which showed no significant effect on transplant growth in our preliminary experiment.

A CO_2-pressured backpack sprayer (Model T; Bellspray) was used to spray the ABA solutions evenly over the seedlings between 1000 and 1100 HR. The sprayer was equipped with three flat-fan nozzles (TP8002VS; TeeJet Technologies) and a CO_2 cylinder with pressure maintained at 276 kPa.

2.2.2.3 Transplant Growth Measurements

Six plants per replication (tray) were randomly selected before the first measurement. All measurements were made at 25, 18, 11, and 1 DBT for 'Excursion II' and at 22, 15, 8, and 1 DBT for 'Colima'.

Stem height and leaf chlorophyll index were repeatedly measured on the selected plants between 8:00 and 10:00 AM at each measurement time. Stem height was measured from the medium surface to the shoot apex. To quantify the elongation speed, relative stem elongation rate (RSER, mm cm^{-1} d^{-1}) was calculated as follows:

$$\text{RSER} = (\ln H_2 - \ln H_1)/(t_2 - t_1) \times 10$$

where $\ln H_1$ and $\ln H_2$ are the natural logarithm of stem height (cm) at time one, t_1, and time two, t_2, respectively.

Leaf chlorophyll index was measured using a chlorophyll meter (SPAD-502; Konica Minolta Sensing) on the youngest fully open leaf and the largest leaf. Two readings were taken per leaf on a leaf lamina between major leaf veins.

At each measurement time, three plants per replication were randomly sampled, and roots were washed to remove the growth medium. Stem diameter was measured immediately below the cotyledonary node using a digital caliper (Absolute Digimatic Caliper Series 500; Mitutoyo, Kawasaki, Japan). The number of cotyledons and true leaves with unfolded laminae and visible petioles were counted. Leaf area was measured using an LI-3100 area meter (LI-COR, Lincoln, NE). Shoots and roots were separated and dried at 65°C for 72 h to determine dry weight.

2.2.2.4 Field Experiment

One d before transplanting, the seedlings were transferred to Texas A&M AgriLife Research and Extension Center (Uvalde, TX) using a customized enclosed trailer equipped with racks to hold up to 70 trays. The transportation was about a 6-h drive and caused no visual damage on the seedlings. Soil at the site was an Uvalde silty clay loam (fine-silty, mixed, hyperthermic Aridic Calciustolls). At pre-plant, the surface (top 18 cm) soil had pH of 7.6, organic matter of 26 g kg^{-1}, and high available macronutrient (P, K, and Mg) levels (> 63 mg kg^{-1}), according to soil tests by Soil, Water and Forage Testing Laboratory at Texas A&M University (College Station, TX).

Seedlings of 'Excursion II' and 'Colima' were transplanted on raised beds (20 cm high and 70 cm wide) in one row per bed on 30 Mar. and 16 Apr. 2010, respectively. A semi-

automatic transplanter (RTME1100; Renaldo Sales & Service, North Collins, NY, USA) was used to control planting depth at the cotyledonary node with 30 cm in-row spacing. Each plot was a 3.7-m long single row with 12 plants. All plots were irrigated through drip tapes (T-Tape 508-12-340; John Deere, Moline, IL) installed at 10 cm depth in the center of each bed. The drip tapes had emitters spaced 30 cm apart, with a flow rate per emitter of 0.77 L h^{-1}. Fertilizers at 120N–37P–100K kg ha^{-1} were applied in six split applications through drip irrigation. Standard pest management practices for peppers were followed.

All field measurements were made periodically from establishment to early harvest. Stem height and leaf chlorophyll index were measured repeatedly on the same plants (four plants per plot) using the criteria described above. Seedling survival was determined on a plot basis using all plants.

Fruits were harvested at the mature green stage seven times during 15 June and 30 July for 'Excursion II' and five times during 29 June and 20 Aug. for 'Colima'. For fruit grading, the grade standards developed by the U.S. Dept. of Agriculture (USDA) for sweet peppers (USDA, 2005) were used for 'Excursion II', and those for hot peppers (USDA, 2007) were used for 'Colima'. Marketable fruits were at least U.S. No. 1 grade, with the minimum size of 6.4 cm wide × 6.4 cm long for 'Excursion II' and 3.8 cm wide × 5 cm long for 'Colima'. Other fruits were graded as unmarketable fruit. Number and fresh weight of marketable and unmarketable fruits were determined.

2.2.2.5 Statistical Design and Analysis

The two cultivars were analyzed separately because of differences in sowing date and measurement schedule. In the greenhouse, five treatments for each cultivar were replicated four

times with one tray per replication in a completely randomized block design. The same experimental design was used in the field.

All data analyses were run in SAS. Unless otherwise noted, *P* values less than 0.05 were considered statistically significant. Treatment effects were tested using the restricted maximum likelihood method with the Kenward–Rogers approximation of degrees of freedom in the MIXED procedure. Pre-treatment data were included as covariates. Two additional tests were run in the MIXED procedure: the Tukey–Kramer test for multiple comparisons of least squares means and orthogonal contrasts for testing two specific hypotheses. The first hypothesis was that all ABA treatments have equivalent growth modulating effects, thereby comparing the control with the pooled ABA treatments. The second hypothesis was that ABA has different effects based on whether it is applied once at 3.8 mM or twice at 1.9 mM, thereby comparing the pooled single-spray treatments with the double-spray treatment. When heteroscedasticity was indicated by a likelihood ratio test, the MIXED procedure was run with the GROUP option in the REPEATED statement.

To assess the linear association between two dependent variables, the data were fit to a simple linear regression model using the REG procedure. The association was considered non-significant when the slope was not significantly different from zero.

2.2.3 Results

2.2.3.1 Stem Height and Diameter

Pre-treatment stem height was 1.2 cm in ‘Excursion II’ and 2.3 cm in ‘Colima’ (Table 2.3). In the control, RSER decreased during the experiment, whereas stem height increased steadily to 11.3 cm in ‘Excursion II’ and 12.3 cm in ‘Colima’. Exogenous ABA inhibited stem elongation similarly in the two cultivars. In ‘Excursion II’, RSER calculated over 7–10 d

following ABA applications at 25, 18, and 11 DBT was reduced by 49% (1.66 vs. 0.85 mm cm^{-1} d^{-1}), 69% (0.91 vs. 0.28 mm cm^{-1} d^{-1}), and 40% (0.46 vs. 0.28 mm cm^{-1} d^{-1}), respectively. In 'Colima', RSER calculated over 7 d following ABA applications at 22, 15, and 8 DBT was reduced by 57% (1.02 vs. 0.44 mm cm^{-1} d^{-1}), 16% (0.91 vs. 0.76 mm cm^{-1} d^{-1}), and 35% (0.38

Table 2.3. Stem height and relative stem elongation rate (RSER) of bell pepper 'Excursion II' and jalapeño 'Colima' seedlings as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 2).

		Stem height (cm)			RSER (mm cm^{-1} d^{-1})		
		DBT					
Cultivar	Treatment[z]	18	11	1	25–18	18–11	11–1
Excursion II	Control	3.87 a[x]	7.31 a	11.3 a	1.66 a	0.91 b	0.46 b
	11 DBT (3.8 mM)	--	--	9.8 b	--	--	0.28 c
	18 DBT (3.8 mM)	--	4.73 b	10.0 b	--	0.28 d	0.75 a
	25 DBT (3.8 mM)	2.19 c	4.56 b	9.0 b	0.85 c	1.07 a	0.69 a
	25 + 18 DBT (1.9 mM)	2.65 b	4.40 b	9.1 b	1.11 b	0.74 c	0.73 a
Orthogonal contrasts[y]		*P* value					
Control vs. ABA		--	--	0.000	--	--	--
ABA ×1 vs. ×2		--	--	0.118	--	--	--
		DBT					
		15	8	1	22–15	15–8	8–1
Colima	Control	4.71 a	9.18 a	12.3	1.02 a	0.91 b	0.38 bc
	8 DBT (3.8 mM)	--	--	10.6	--	--	0.25 c
	15 DBT (3.8 mM)	--	7.75 b	11.1	--	0.76 c	0.52 ab
	22 DBT (3.8 mM)	3.12 c	7.28 b	11.0	0.44 c	1.22 a	0.59 a
	22 + 15 DBT (1.9 mM)	3.38 b	7.54 b	10.9	0.56 b	1.14 a	0.53 ab
Orthogonal contrasts		*P* value					
Control vs. ABA		--	--	0.017	--	--	--
ABA ×1 vs. ×2		--	--	0.940	--	--	--

DBT, days before transplanting.

[z]Treatments were as follows: no spray control, three timings of a single spray with 3.8 mM ABA, and one treatment of a double spray with 1.9 mM ABA. Spray volume of 0.61 L m^{-2} (0.71 ml/plant) was used for all ABA treatments.

[y]Orthogonal contrasts tested two hypotheses: control vs. all ABA treatments (control vs. ABA) and all single-spray treatments vs. double-spray treatment (ABA ×1 vs. ×2).

[x]For each cultivar, least squares means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Pretreatment stem height was 1.2 cm at 25 DBT in 'Excursion II' and 2.3 cm at 22 DBT in 'Colima'.

vs. 0.25 mm cm^{-1} d^{-1}), respectively. During the subsequent measurement periods, however, the ABA treatments showed higher RSER than the control by up to 63% in 'Excursion II' (0.46 vs. 0.75 mm cm^{-1} d^{-1} at 11–1 DBT) and 57% in 'Colima' (0.38 vs. 0.59 mm cm^{-1} d^{-1} at 8–1 DBT). As a result, the magnitude of height control became gradually smaller; stem height reductions by ABA in 'Excursion II' were 31% to 43% at 18 DBT (3.87 vs. 2.19 to 2.65 cm), 35% to 40% at

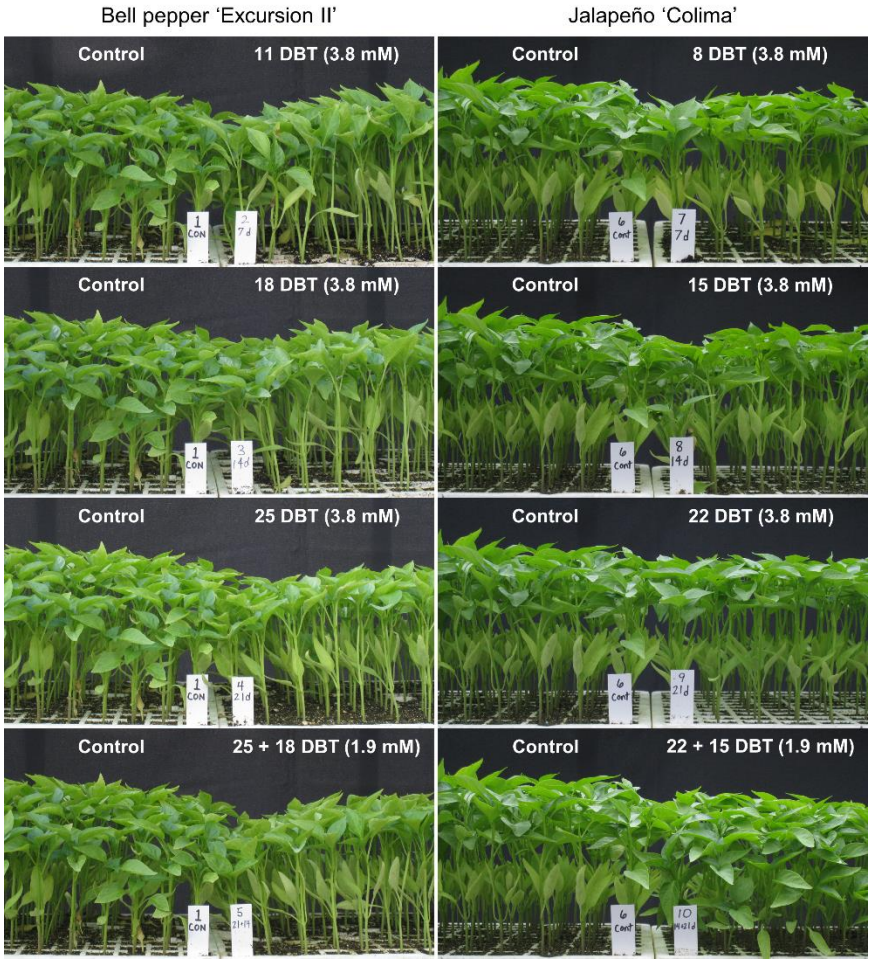

Fig. 2.10. Bell pepper 'Excursion II' and jalapeño 'Colima' seedlings 0 and 1 d before transplanting (DBT), respectively (Study 2). Treatments were as follows: no spray control, three timings of a single spray with 3.8 mM abscisic acid (ABA), and one treatment of a double spray with 1.9 mM ABA. Spray volume of 0.61 L m^{-2} (0.71 ml/plant) was used for all ABA treatments. Height and leaf area reductions by ABA were apparent in most treatment trays. Cotyledon chlorosis and abscission were most readily noticeable in the 8 DBT treatment of 'Colima'.

11 DBT (7.31 vs. 4.40 to 4.73 cm), and 12% to 20% at 1 DBT (11.3 vs. 9.0 to 10.0 cm), and those in 'Colima' were 28% to 34% at 15 DBT (4.71 vs. 3.12 to 3.38 cm), 18% to 21% at 8 DBT (9.18 vs. 7.28 to 7.75 cm), and 10% to 14% at 1 DBT (12.3 vs. 10.6 to 11.1 cm). These reductions were statistically significant, except at 1 DBT in 'Colima', when significance was indicated only by orthogonal contrasts comparing the control with the average of all ABA treatments (12.3 vs. 10.9 cm). Among the ABA treatments, neither multiple comparisons nor orthogonal contrasts detected a significant difference in final stem height of both cultivars. The height control effect of ABA was readily visible in most treatment trays (Fig. 2.10).

Stem diameter measured at 1 DBT showed different responses to ABA in the two cultivars (Fig. 2.11). Stem diameter of 'Excursion II' was unaffected by ABA, ranging from 2.39 to 2.45 mm, whereas that of 'Colima' was 13% smaller in the 22 DBT treatment than in the control (2.70 vs. 2.36 mm). In 'Colima', although other ABA treatments were not significant, the

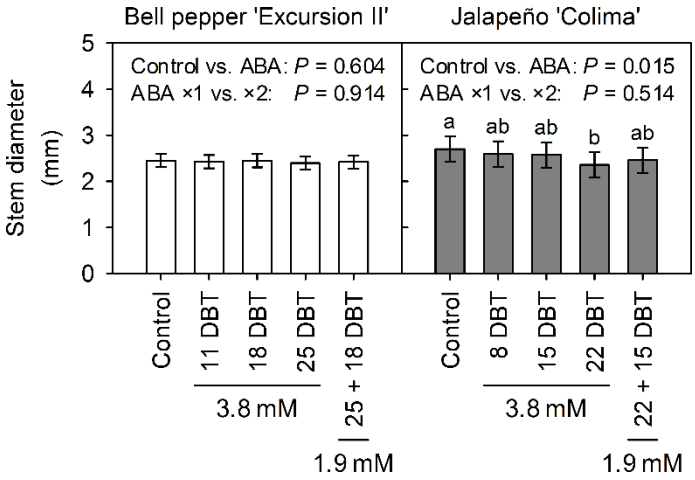

Fig. 2.11. Stem diameter of bell pepper 'Excursion II' (open bar) and jalapeño 'Colima' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 2). Treatments are as described in Fig. 2.10. Data are least squares means ± 95% confidence intervals (n = 4). Means with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Orthogonal contrasts tested two hypotheses: control vs. all ABA treatments (control vs. ABA) and all single-spray treatments vs. double-spray treatment (ABA ×1 vs. ×2).

pooled ABA treatments had significantly smaller stem diameter than the control (2.70 vs. 2.49 mm).

2.2.3.2 Leaf Number and Area

Cotyledon abscission occurred in the 11 and 18 DBT treatments of 'Excursion II' and in the 8 and 22 DBT treatments of 'Colima' (Fig. 2.10), causing non-significant reductions in cotyledon number and area at 1 DBT (Fig. 2.12A and B). In other treatments, both cotyledons remained intact until 1 DBT (Fig. 2.12A).

True leaves measured at 1 DBT showed similar responses to ABA in the two cultivars; exogenous ABA had no significant effect on leaf number (Fig. 2.12A), whereas it inhibited leaf

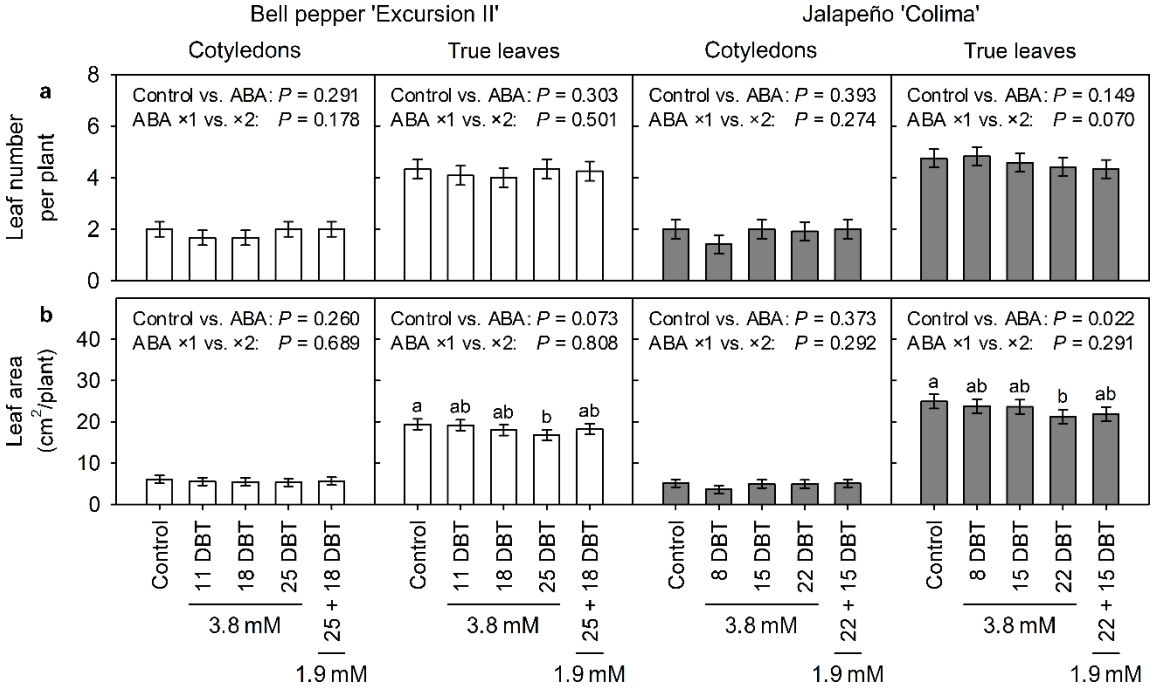

Fig. 2.12. Leaf growth of bell pepper 'Excursion II' (open bar) and jalapeño 'Colima' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 2): (**A**) leaf number and (**B**) leaf area. Treatments and statistical comparisons are as described in Fig. 2.10 and Fig. 2.11, respectively.

expansion (Fig. 2.12B). The inhibitory effect was significant in the 25 DBT treatment of 'Excursion II' and in the 22 DBT treatment of 'Colima', reducing leaf area by 13% (19.4 vs. 16.8 cm^2) and 15% (24.9 vs. 21.3 cm^2), respectively, compared with the corresponding controls. In 'Colima', the pooled ABA treatments also had significantly lower leaf area than the control (24.9 vs. 18.1 cm^2). Leaf area reductions by ABA were readily visible in most treatment trays (Fig. 2.10).

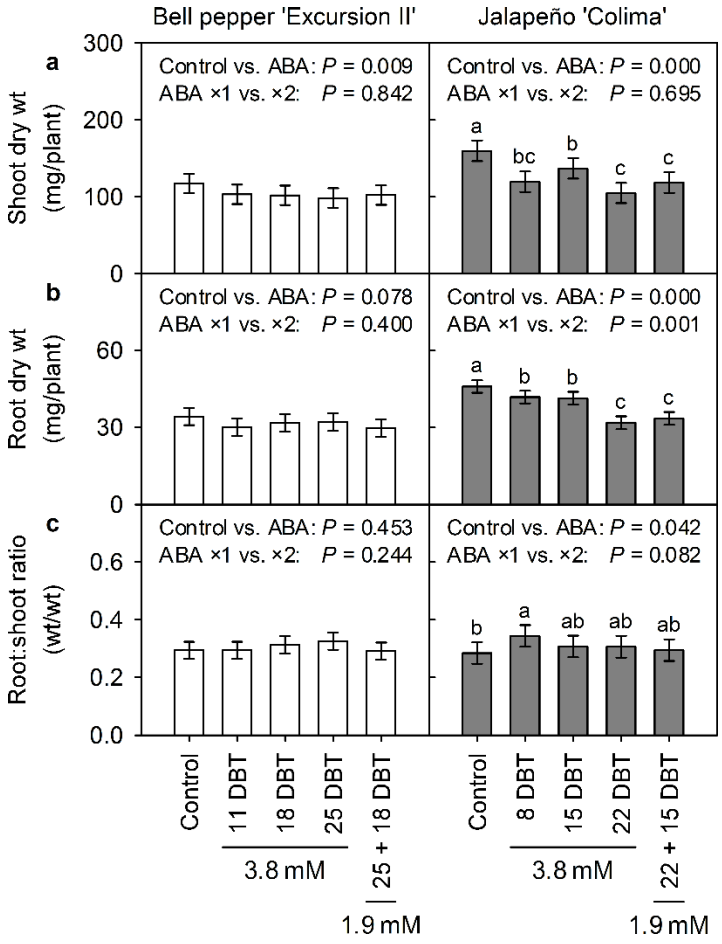

Fig. 2.13. Dry matter accumulation and partitioning of bell pepper 'Excursion II' (open bar) and jalapeño 'Colima' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 2): (**A**) shoot dry weight, (**B**) root dry weight, and (**C**) root-to-shoot ratio. Treatments and statistical comparisons are as described in Fig. 2.10 and Fig. 2.11, respectively.

2.2.3.3 Dry Matter Accumulation and Partitioning

Dry matter accumulation and partitioning measured at 1 DBT showed different responses to ABA in the two cultivars (Fig. 2.13A–C). In 'Excursion II', shoot dry weight was 12% to 16% smaller in the ABA treatments (98 to 103 mg) than in the control (117 mg) (Fig. 2.13A). These reductions were not significant, except when all ABA treatments were pooled (102 mg) by orthogonal contrasts. A similar trend ($P = 0.078$) was found in root dry weight, with 6% to 13% reductions by ABA (Fig. 2.13B). Root-to-shoot ratio was unaffected by ABA, ranging from 0.29 to 0.33 (Fig. 2.13C). Shoot dry weight was positively correlated with leaf area ($r^2 = 0.49$) and stem height ($r^2 = 0.21$), whereas it had no significant correlation with stem diameter (Fig. 2.14).

In 'Colima', shoot and root dry matter accumulation was inhibited by all ABA treatments (Fig. 2.13A–C). The 22 DBT treatment had the strongest inhibition, reducing shoot and root dry weight by 34% (160 vs. 105 mg) and 31% (46.0 vs. 31.9 mg), respectively (Fig. 2.13A and B). The equivalent dry weight reductions were also observed in the 22 + 15 DBT treatment. For roots, as other two ABA treatments (8 and 15 DBT) were less inhibitive than the 22 + 15 DBT treatment, the pooled single-spray treatments had significantly higher dry weight than the double-spray treatment (38.0 vs. 33.6 mg) (Fig. 2.13B). The magnitude of shoot and root dry weight reductions was similar, except for the 8 DBT treatment, in which roots were relatively less inhibited. Consequently, this treatment had 21% higher root-to-shoot ratio than the control (0.28 vs. 0.34) (Fig. 2.13C). Although other ABA treatments were not significant, the pooled ABA treatments (0.31) had significantly higher root-to-shoot ratio than the control. Shoot dry weight was positively correlated with leaf area ($r^2 = 0.76$) and stem diameter ($r^2 = 0.42$), whereas it had no significant correlation with stem height (Fig. 2.14).

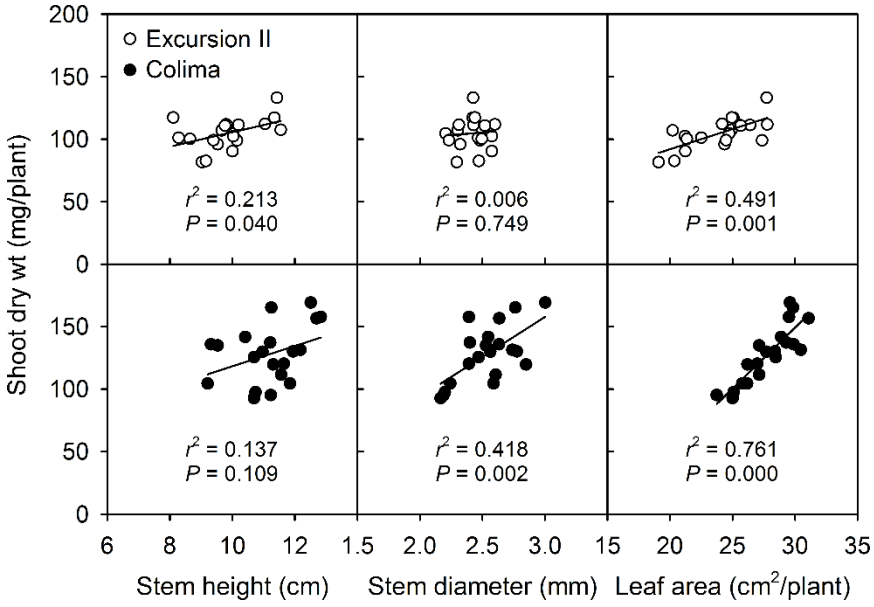

Fig. 2.14. Linear correlations between shoot dry weight and other growth variables of bell pepper 'Excursion II' (open symbol) and jalapeño 'Colima' (black symbol) seedlings 1 d before transplanting (Study 2). Treatments are as described in Fig. 2.10.

2.2.3.4 Leaf Chlorosis

Leaf chlorophyll index measured at 1 DBT showed different responses to ABA in the two cultivars (Fig. 2.15). In 'Excursion II', leaf chlorophyll index of the youngest fully open leaf was unaffected by ABA, whereas that of the largest leaf was significantly higher in the 18 DBT treatment (27.9) than in the control (23.1) and the 11 DBT treatment (22.8). In 'Colima', by contrast, leaf chlorophyll index of the largest leaf was unaffected by ABA, whereas that of the youngest fully open leaf was 5% to 11% lower in the ABA treatments (33.0 to 35.2) than in the control (37.1). These reductions were not significant, except when all ABA treatments were pooled (34.0) by orthogonal contrasts. Chlorosis was noticeable especially on the cotyledons in the 8 DBT treatment of 'Colima' (Fig. 2.10).

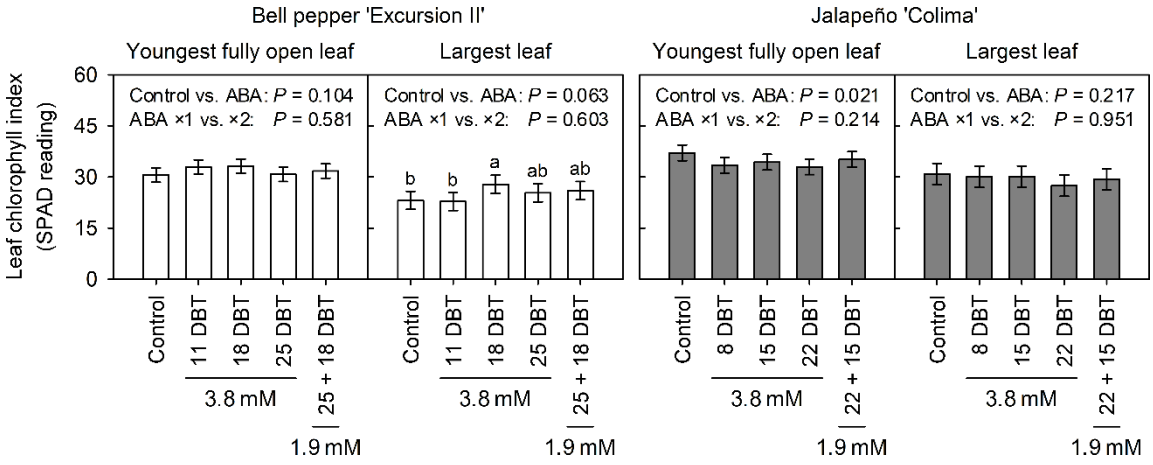

Fig. 2.15. Leaf chlorophyll index of bell pepper 'Excursion II' (open bar) and jalapeño 'Colima' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 2). Treatments and statistical comparisons are as described in Fig. 2.10 and Fig. 2.11, respectively.

2.2.3.5 Field Growth and Yield

From 1 DBT to 24 DAT, stem height decreased by 0.4 to 2.3 cm in 'Excursion II' and by 1.2 to 2.2 cm in 'Colima' (Tables 2.3 and 2.4). These reductions were due to the planting depth set at the cotyledonary node, which averaged 3.0 cm in 'Excursion II' and 2.6 cm in 'Colima' (data not shown). At 24 DAT, except the 11 DBT treatment of 'Excursion II' and the 22 DBT treatment of 'Colima', the ABA-treated plants were significantly shorter than the control plants by 24% to 30% in 'Excursion II' and by 13% to 19% in 'Colima' (Table 2.4). Orthogonal contrasts also found significant differences in two additional hypothesis tests. First, the pooled ABA treatments had shorter stem height than the control in both cultivars. Second, the pooled single-spray treatments had taller stem height than the double-spray treatment in 'Excursion II' with $P = 0.054$ (8.1 vs. 7.4 cm) and in 'Colima' with $P = 0.008$ (9.5 vs. 8.7 cm). As more rapid stem elongation occurred thereafter, the magnitude of growth inhibition by ABA became smaller and non-significant in both cultivars.

Table 2.4. Post-transplanting stem elongation of bell pepper 'Excursion II' and jalapeño 'Colima' from establishment to early harvest as affected by abscisic acid (ABA) applied during transplant growth as a single high dose or repeated low doses (Study 2).

		Stem height (cm)			
		DBT			
Cultivar	Treatment[z]	24	45	66	94
Excursion II	Control	10.2 a[x]	16.4	36.1	53.0
	11 DBT (3.8 mM)	9.4 a	13.6	33.3	53.6
	18 DBT (3.8 mM)	7.8 b	13.7	35.4	53.4
	25 DBT (3.8 mM)	7.1 b	13.8	35.5	54.8
	25 + 18 DBT (1.9 mM)	7.4 b	14.7	34.7	54.7
Orthogonal contrasts[y]		*P* value			
Control vs. ABA		0.000	0.022	0.494	0.640
ABA ×1 vs. ×2		0.054	0.362	0.998	0.749
		DBT			
		24	46	67	108
Colima	Control	10.7 a	18.2	34.4	72.8
	8 DBT (3.8 mM)	9.4 bc	16.1	34.0	70.4
	15 DBT (3.8 mM)	9.4 bc	17.5	34.8	74.6
	22 DBT (3.8 mM)	9.7 ab	18.0	35.8	69.6
	22 + 15 DBT (1.9 mM)	8.7 c	15.1	33.0	71.4
Orthogonal contrasts		*P* value			
Control vs. ABA		0.000	0.178	0.981	0.525
ABA ×1 vs. ×2		0.008	0.080	0.347	0.961

DBT, days before transplanting.
[z]Treatments are as described in Table 3.
[y]Orthogonal contrasts are as described in Table 3.
[x]For each cultivar, least squares means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

For seedling survival rate and leaf chlorophyll index, neither multiple comparisons nor orthogonal contrasts detected a significant difference in both cultivars throughout the experiment (data not shown). Seedling loss was minimal (< 5%) in all treatments.

Marketable yield showed no significant difference among the treatments, averaging 13.6 t ha^{-1} with 3.6 fruits per plant for 'Excursion II' and 30.3 t ha^{-1} with 46 fruits per plant for 'Colima' (Table 5). Most of other yield variables were also unaffected by ABA. When all ABA

treatments were pooled, they had significantly heavier fruit size than the control in 'Excursion II' (112 vs. 119 g), but this difference was practically negligible.

Table 2.5. Yield components of bell pepper 'Excursion II' and jalapeño 'Colima' from establishment to early harvest as affected by abscisic acid (ABA) applied during transplant growth as a single high dose or repeated low doses (Study 2).

		Marketable yield			Total yield
Cultivar	Treatment[z]	(Fruit no./plant)	(g/fruit)	(t ha^{-1})	(t ha^{-1})
Excursion II	Control	3.8	112.4	14.2	18.4
	11 DBT (3.8 mM)	3.4	117.7	12.8	16.6
	18 DBT (3.8 mM)	3.3	116.7	12.3	16.6
	25 DBT (3.8 mM)	3.4	120.5	13.1	18.3
	25 + 18 DBT (1.9 mM)	4.0	120.7	15.5	20.1
Orthogonal contrasts[y]			*P* value		
Control vs. ABA		0.515	0.014	0.680	0.807
ABA ×1 vs. ×2		0.199	0.370	0.161	0.209
Colima	Control	45.5	20.8	29.5	31.5
	8 DBT (3.8 mM)	44.1	21.5	30.0	32.7
	15 DBT (3.8 mM)	51.2	20.8	33.0	35.2
	22 DBT (3.8 mM)	46.2	21.4	30.9	33.3
	22 + 15 DBT (1.9 mM)	43.1	20.3	27.9	30.6
Orthogonal contrasts			*P* value		
Control vs. ABA		0.866	0.687	0.744	0.622
ABA ×1 vs. ×2		0.292	0.149	0.235	0.307

DBT, days before transplanting.
[z]Treatments are as described in Table 3.
[y]Orthogonal contrasts are as described in Table 3.

2.2.4 Discussion

2.2.4.1 Cultivar-dependent Growth Modulation by Abscisic Acid

The two pepper cultivars, bell pepper 'Excursion II' and jalapeño 'Colima', showed different growth responses to foliar spray of ABA. First, only 'Excursion II' maintained significant height reductions by all ABA treatments until the transplanting stage, ranging from

12% to 20%. The magnitude of height suppression is comparable to that previously reported for ABA spray and drench treatments in other cultivars of bell pepper seedlings (Biai et al., 2011; Leskovar and Cantliffe, 1992). Mechanical conditioning is another strategy with similar effects. For example, Björkman (1998) reported that stem height of tomato seedlings was reduced by 20% when the upper canopy was brushed 10 strokes per day.

Stocky and strong transplants are generally characterized by thick stems. However, stem diameter of 'Colima' was rather reduced by ABA, suggesting that, in jalapeño, ABA may weaken stem strength and thus limit the benefit of height control. This effect is a drawback to ABA treatments compared with mechanical stimulation, which can both shorten and thicken stems by stimulating ethylene production (Garner and Björkman, 1996; Garner and Björkman, 1997; Hiraki and Ota, 1975; Latimer, 1998).

Another negative side effect of ABA in 'Colima' is the strong inhibition of shoot and, more importantly, root biomass accumulation. For vegetable transplants, large root systems are necessary not only to improve stand establishment (Agehara and Leskovar, 2012), but also to facilitate pulling of transplants from trays (Vavrina, 2002). In this cultivar, shoot biomass reductions were associated with reductions in stem diameter and leaf area, suggesting that overall growth delay was induced by ABA. Such growth delay is not desirable for commercial nurseries because it prolongs the transplant production cycle and increases the cost of production. Conversely, it may be of value as a growth holding strategy when transplanting is delayed because of inclement weather at the time of field establishment.

Furthermore, leaf chlorosis, a sign of poor transplant quality, was induced by ABA in 'Colima', as indicated by visual symptoms and reductions in leaf chlorophyll index. The ABA-induced chlorosis can be attributed to the senescing effects of ABA, resulting from the gene

expression of hydrolytic enzymes involved in chlorophyll breakdown (Weaver et al., 1998) or the stimulation of ethylene production (Gepstein and Thimann, 1981).

These results suggest that for 'Excursion II', foliar spray of ABA is effective in producing stocky transplants with minimal negative side effects. Similar cultivar-dependent effectiveness of ABA has been reported. For transplant height suppression, Biai et al. (2011) found that drench application of ABA was most effective in bell pepper 'Aristotle', intermediate in banana pepper 'Pageant', and non-significant in jalapeño 'Grande'. Therefore, sweet pepper cultivars may be more suited for the height control effect of ABA than hot pepper cultivars.

2.2.4.2 Age-dependent Sensitivity to Abscisic Acid

Abscisic acid functions differently depending on tissue type in plants (Taiz and Zeiger, 2010). Therefore, to optimize its application timing for effective height control, age-dependent sensitivity must be considered not only for plant height but also for other growth components. At the transplanting stage, age-dependent sensitivity to ABA was evident in leaf area of both cultivars, and in stem diameter and shoot and root biomass of 'Colima'. In all cases, growth inhibition was maximal when 3.8 mM ABA was applied at the cotyledon stage, during which relative growth rate was most rapid. The inhibition of these growth variables are generally negative characteristics for vegetable transplants as described above. To minimize quality loss, therefore, ABA should be applied at least one week after the emergence of first true leaf. This recommendation is different from that by Biai et al. (2011) to the extent that they use transplant height as a sole indicator and suggest initiating ABA application at the cotyledon stage.

2.2.4.3 Single vs. Double Application of Abscisic Acid

Because excessively high concentrations of ABA can induce undesirable side effects, such as leaf chlorosis and abscission (Agehara and Leskovar, 2012; Kim and van Iersel, 2011; Waterland et al., 2010c), repeated application of low doses may be a more effective strategy than applying a single high-dose. Among the variables measured in this study, statistical differences between single- and double-application of ABA were minimal. This observation suggests that the effectiveness of ABA in height control can be easily adjusted by changing the concentration and number of application. One practically significant advantage of repeated low-dose application may be a reduced risk of cotyledon abscission.

2.2.4.4 Effects of Abscisic Acid on Field Growth and Yield Are Minimal

Field performance must be evaluated to justify the advantages of transplant growth modification at nurseries. Except for the relatively slow stem elongation during early field establishment, the ABA-treated plants had similar field growth and yield compared with the control plants. Their initial slow growth could be due simply to insufficient leaf area or root system to support the new growth.

It is important to note that all transplants used in the field experiment were shipped from the nursery in trays and thus were minimally damaged. In common commercial operations, mechanical injury often occurs when transplants are pulled from trays and packed in boxes at high density for shipment (Cantliffe, 1993). Leskovar and Cantliffe (1991) reported that tomato transplants stored in trays produced more extra-large fruit than transplants packed in boxes. Risse et al. (1985) shipped tomato plants packed at 1000 or 1250 plants per crate from Georgia to Ohio (USA), and found that plant survival and yield were reduced by dense packing. Therefore, our field data may not reflect the advantage of height suppression to minimize

damage during commercial shipping operations. Nevertheless, this study demonstrates that, with only transient growth modulation and no negative impact on marketable yield, ABA is an effective height control tool for sweet pepper transplants. To maximize the benefits with minimal negative side effects, the application strategy must be optimized based on both cultivar- and age-dependent effectiveness.

2.3 Study 3: Growth Reductions by Exogenous Abscisic Acid Limit the Benefit of Height Control in Diploid and Triploid Watermelon Transplants

Height control is important to produce compact vegetable transplants that are suitable for shipping and transplanting. Although abscisic acid inhibits stem elongation, it can also induce other growth modifications. To optimize its application timing for effective height control, age-dependent sensitivity of various growth variables to ABA was examined in diploid 'Summer Flavor 800' and triploid 'Summer Sweet 5244' watermelon. Seedlings were sprayed once with 1.9 mM ABA at 25, 18, or 11 DBT, or twice with 0.95 mM ABA at 25 and 18 DBT. The application rate was 0.55 mg ABA per plant with the spray volume of 0.61 $L \cdot m^{-2}$ (1.1 ml/plant). Only the single-spray treatment at 25 DBT (cotyledon stage) suppressed plant height by inhibiting petiole elongation. This effect was similar in both cultivars, with 13% to 14% reductions at the transplanting stage compared with the untreated control. Undesirable growth modifications were also induced by ABA. In both cultivars, all ABA treatments caused 16% to 23% shoot biomass reductions mainly by inhibiting leaf expansion. Additionally, ABA treatments reduced stem diameter and root biomass in 'Summer Flavor 800'. The double-spray treatment had similar growth-modulating effects as the single-spray treatments, except that it induced cotyledon abscission in 'Summer Flavor 800'. These results suggest that although ABA

applied at the cotyledon stage can reduce watermelon transplant height, the benefit is limited because of overall growth reductions, which can occur regardless of application timing. On the other hand, in triploid 'Summer Sweet 5244', moderate shoot growth delay by ABA may be of value as a growth holding strategy when transplanting is delayed because of inclement weather at the time of field establishment. Importantly, field evaluations demonstrated that the growth modulation by ABA is only transient with no negative impact on marketable yield and fruit quality.

2.3.1 Introduction

Vegetable transplant production in high-density plug trays can induce excessive stem elongation as a result of shade avoidance responses (Marr and Jirak, 1990; Smith, 1994). The resulting spindly transplants are generally considered unsuitable for shipping and transplanting, as they are susceptible to damage during these operations (Garner and Björkman, 1996; Shaw, 1993) and to wind damage in the field (Garner and Björkman, 1999; Latimer and Mitchell, 1988). Consequently, their field establishment can be slow and non-uniform, potentially delaying early harvest and limiting marketable yield.

Height control is important for producing compact and high quality vegetable transplants. Although several gibberellin inhibitors, such as daminozide, paclobutrazol, and uniconazole, are commercially used to produce compact plants in ornamentals and flowers (Gibson and Whipker, 2001; Whipker et al., 2000), they tend to have long-term growth inhibitory effects (Cantliffe, 1993; Latimer, 1991) and only uniconazole is currently registered for vegetable crops. Furthermore, the approved vegetables are limited mostly to solanaceous crops, including eggplant, pepper, and tomato. Alternatively, stem elongation can be reduced by mechanical stimulation, such as brushing the upper canopy, shaking, and vibration by wind or

forced aeration (Baden and Latimer, 1992; Björkman, 1999; Garner and Björkman, 1997). These mechanical conditioning methods inhibit stem elongation by stimulating ethylene production, which in turn inhibits cell elongation and promotes stem thickening (Hiraki and Ota, 1975; Zarembinski and Theologis, 1994). However, their commercial application is limited by high costs of automation and labor (Latimer, 1998).

Abscisic acid can act as a physiological inhibitor of stem elongation in some vegetable transplants, including pepper, eggplant, tomato, and cucumber (Biai et al., 2011; Latimer and Mitchell, 1988; Yamazaki et al., 1995). In contrast to gibberellin inhibitors, ABA can be rapidly inactivated in plant tissues by oxidation or conjugation (Davies and Jones, 1991), suggesting that it may be more suitable for vegetable transplants because of its transient growth inhibitory effects. The potential of ABA as a height control agent has been evaluated mainly in bell pepper seedlings. For example, Leskovar and Cantliffe (1992) reported that the concentration effect of ABA on stem elongation was quadratic, with height suppression occurring above 10 μM. Biai et al. (2011) suggested that the effectiveness of height control by ABA is age-dependent, and that ABA application should be initiated at the cotyledon stage. However, this recommendation is based solely on plant height, although other growth components are also known to be affected by ABA (Taiz and Zeiger, 2010). Moreover, high-dose applications of ABA have negative side-effects, such as leaf chlorosis and abscission (Agehara and Leskovar, 2012; Kim and van Iersel, 2011; Waterland et al., 2010c). Therefore, the overall growth modification must be considered to further optimize ABA application methods for height control.

Seedless (triploid) watermelon is generally the most expensive vegetable to produce transplants, mainly because of the high cost of seeds, low seedling vigor (Grange et al., 2003), and extra care required for transplant production (Vavrina, 2002). Nonetheless, this highly valuable crop has been neither approved for the use of uniconazole, the only growth regulator

currently available for height control of vegetable transplants, nor tested for ABA responses. The first objective of this study was, therefore, to examine the age-dependent sensitivity of various growth variables to ABA in diploid and triploid watermelon seedlings under greenhouse conditions. This information will be useful to determine the optimal application timing for the most effective height control. The second objective was to evaluate if the advantages of height control in ABA-treated transplants would be translated in improved field performance.

2.3.2 Materials and Methods

2.3.2.1 Plant Material and Growth Conditions

Seeds of two major watermelon cultivars in Texas, diploid 'Summer Flavor 800' and triploid 'Summer Sweet 5244' (Abbott & Cobb), were sown on 16 Feb. 2010 in a polystyrene tray with 128 inverted pyramid cells each containing 35 mL of peat-lite mix (Speedling Peat-lite; Speedling). Seedlings were grown at a commercial nursery greenhouse (Speedling) located in Alamo, TX until they reached the optimal size for transplanting according to the nursery's commercial standard (typically 13–15 cm). Average daily air temperature during seedling growth ranged from 9 to 26 °C.

2.3.2.2 Abscisic Acid Treatments

There were five treatments for each cultivar: no spray control, three timings of single spray with 1.9 mM (500 $mg \cdot L^{-1}$) ABA, and one treatment of double spray with 0.95 mM (250 $mg \cdot L^{-1}$) ABA. The single spray was performed at 25, 18, or 11 DBT (17, 24, or 31 d after sowing), and the double spray was performed at 25 and 18 DBT. Seedlings had fully expanded cotyledons with one or two immature true leaves at the time of first ABA application. Spray volume was set at 0.61 $L \cdot m^{-2}$ (1.1 ml/plant), which wetted the leaves thoroughly to the dripping

point. The application rate was 0.55 mg ABA per plant in all ABA treatments. These spray concentrations and volumes were selected based on the results in Study 1-1.

The formulation of ABA stock solution was VBC-30151 containing 10% of S-ABA, a naturally occurring active form in plants. Test solutions were prepared immediately before each treatment by diluting the stock solution with irrigation water at the nursery. CapSil was added at 0.05% (v/v) as an adjuvant according to the manufacture's protocol. This adjuvant showed no significant effect on transplant growth in our preliminary experiment.

A CO_2-pressurized backpack sprayer (Model T; Bellspray) was used to spray the ABA solutions evenly over the seedlings between 1000 and 1100 HR. The sprayer was equipped with three flat-fan nozzles (TP8002VS; TeeJet Technologies) and a CO_2 cylinder with pressure maintained at 276 kPa.

2.3.2.3 Transplant Growth Measurements

All measurements were made at 25, 18, 11, and 1 DBT. Non-destructive measurement variables include stem and shoot height, petiole length, and leaf chlorophyll index, and destructive measurement variables include stem diameter, leaf number, leaf area, and shoot and root dry weight.

Six plants per replication (tray) were randomly selected before the first measurement. All non-destructive measurements were made repeatedly on the selected plants between 0800 and 1000 HR on each day. Stem height was measured from the medium surface to the shoot apex, and shoot height was measured up to the highest leaf tip by stretching the leaves. The length of the longest petiole was measured from the node to the leaf attachment point. Relative stem elongation rate (RSER, $mm \cdot cm^{-1} \cdot d^{-1}$) was calculated as follows:

$$RSER = (\ln H_2 - \ln H_1)/(t_2 - t_1) \times 10$$

where $\ln H_1$ and $\ln H_2$ are the natural logarithm of stem height (cm) at time one, t_1, and time two, t_2, respectively.

Leaf chlorophyll index was measured using a chlorophyll meter (SPAD-502; Konica Minolta Sensing) on two leaves differing in maturity, the youngest fully open leaf and the largest leaf. Two readings were taken per leaf on a leaf lamina between major leaf veins.

At each measurement time, three plants per replication were randomly sampled, and roots were washed to remove the growth medium. Stem diameter was measured immediately below the cotyledonary node using a digital caliper (Absolute Digimatic Caliper Series 500; Mitutoyo). The number of cotyledons and true leaves with unfolded laminae and visible petioles were counted. Leaf area was measured using an LI-3100 area meter (LI-COR). Shoots and roots were separated and dried at 65°C for 72 h to determine dry weight.

2.3.2.4 Field Experiment

One d before transplanting, the seedling trays were transported in an enclosed trailer to Texas A&M AgriLife Research and Extension Center in Uvalde, TX. Soil at the site was an Uvalde silty clay loam (fine-silty, mixed, hyperthermic Aridic Calciustolls). At pre-plant, the surface (top 18 cm) soil had pH of 7.6, organic matter of 26 $g \cdot kg^{-1}$, and high available macronutrient (P, K, and Mg) levels (> 63 $mg \cdot kg^{-1}$), according to soil tests by the Soil, Water and Forage Testing Laboratory at Texas A&M University in College Station, TX.

Seedlings were transplanted on raised beds (20 cm high and 1.6 m wide) in one row per bed on 30 Mar, 2010. A semi-automatic transplanter (RTME1100; Renaldo Sales & Service) was used to control planting depth at the cotyledonary node with 91 cm in-row spacing. Each plot was an 11 m long single row with 12 plants. There were 480 plants in total with a 1:1 diploid to triploid ratio, providing sufficient pollinizers (diploid watermelon) for optimum

pollination of triploid watermelon plants. Pollinator bees were not used because native bees generally provide adequate pollination at the experiment site. All plots were irrigated through drip tapes (T-Tape 508-12-340; John Deere) installed at 10 cm depth in the center of each bed. The drip tapes had emitters spaced 30 cm apart, with a flow rate per emitter of 0.77 $L \cdot h^{-1}$. Fertilizers at 80N–45P–17K $kg \cdot ha^{-1}$ were applied in five split applications through the subsurface drip system. Standard pest management practices for watermelon were followed.

All field measurements were made repeatedly on the same plants (four plants per plot) from establishment to early harvest. Vine length was measured from the soil surface to the shoot apex. Leaf chlorophyll index were measured using the same method used in the greenhouse. Seedling survival was determined on a plot basis using all plants.

Fruits were harvested on 1 and 14 July and graded based on the USDA grade standards (USDA, 2006) . Marketable fruits were at least U.S. No. 1 grade with a minimum size of 4.54 kg. Other fruits were graded as unmarketable fruit. Number and fresh weight of marketable and unmarketable fruits were determined. Among the marketable fruits harvested on 1 July (peak harvest), three fruits per plot were sampled and cut transversely along the equator for quality assessment. Mesocarp firmness was measured using a digital force meter (DFM10; AMETEK, Largo, FL) with an 11 mm diameter round-head probe. Soluble solids content was measured using a digital refractometer (PR-101; Atago, Tokyo, Japan) on unfiltered juice squeezed from the mesocarp tissue. Three and two readings were taken per fruit for firmness and soluble solids content, respectively.

2.3.2.5 Statistical Design and Analysis

In the greenhouse, five treatments for each cultivar were replicated four times with one tray per replication in a completely randomized block design. The same experimental design was

used in the field. The two cultivars could not be compared at the same development stage because of relatively slow germination and early seedling growth of 'Summer Sweet 5244', which are typical to triploid watermelon (Grange et al., 2003; Hodges, 2007). Consequently, the two cultivars were analyzed separately.

All data analyses were run in SAS, and *P* values less than 0.05 were considered statistically significant. Treatment effects were tested using the restricted maximum likelihood method with the DDFM=KR option in the MIXED procedure. Pre-treatment data were included as covariates. Two additional tests were run in the MIXED procedure: the Tukey–Kramer test for multiple comparisons of least squares means and orthogonal contrasts for testing two specific hypotheses. The first hypothesis was that all ABA treatments have equivalent growth modulating effects, thereby comparing the control with the pooled ABA treatments. The second hypothesis was that ABA has different effects based on whether it is applied once at 1.9 mM or twice at 0.95 mM, thereby comparing the pooled single-spray treatments with the double-spray treatment. When heteroscedasticity was indicated by a likelihood ratio test, the MIXED procedure was run with the GROUP option in the REPEATED statement.

To assess the linear association between two dependent variables, the data were fit to a simple linear regression model using the REG procedure. The association was considered non-significant when the slope was not significantly different from zero.

2.3.3 Results

2.3.3.1 Stem and Shoot Height

Pre-treatment stem height was 1.97 cm in 'Summer Flavor 800' and 2.41 cm in 'Summer Sweet 5244' (Table 2.6). In the control, RSER decreased during the experiment (Table

2.7), whereas stem height increased steadily to 3.96 cm in 'Summer Flavor 800' and 4.22 cm in 'Summer Sweet 5244' (Table 2.6). Exogenous ABA inhibited stem elongation similarly in the two cultivars. In 'Summer Flavor 800', RSER calculated over 7–10 d following ABA applications at 25, 18, and 11 DBT was reduced by 47% (0.66 vs. 0.35 $mm \cdot cm^{-1} \cdot d^{-1}$), 54% (0.14 vs. 0.06 $mm \cdot cm^{-1} \cdot d^{-1}$), and 17% (0.14 vs. 0.11 $mm \cdot cm^{-1} \cdot d^{-1}$), respectively (Table 2.7). In

Table 2.6. Stem and shoot height of diploid 'Sumer Flavor 800' and triploid 'Summer Sweet 5244' watermelon seedlings as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 3).[z]

		Stem height (cm)				Shoot height (cm)
		DBT[x]				
Cultivar[z]	Treatment[y]	25	18	11	1	1
SF 800	Control	1.97	3.14 a[v]	3.43 a	3.96	15.7 a
	11 DBT (1.9 mM)	--	--	--	3.85	15.9 a
	18 DBT (1.9 mM)	--	--	3.32 ab	3.96	15.7 a
	25 DBT (1.9 mM)	--	2.44 b	3.13 b	3.58	13.5 b
	25 + 18 DBT (0.95 mM)	--	2.90 a	3.33 ab	3.77	15.0 ab
Orthogonal contrasts[w]		*P* value				
Control vs. ABA		--	--	--	0.226	0.168
ABA ×1 vs. ×2		--	--	--	0.860	0.934
SS 5244	Control	2.41	3.67 a	3.93 a	4.22	14.0 a
	11 DBT (1.9 mM)	--	--	--	4.16	14.3 a
	18 DBT (1.9 mM)	--	--	3.91 ab	4.20	13.1 ab
	25 DBT (1.9 mM)	--	2.90 c	3.56 b	3.67	12.2 b
	25 + 18 DBT (0.95 mM)	--	3.36 b	3.85 ab	4.17	12.8 ab
Orthogonal contrasts		*P* value				
Control vs. ABA		--	--	--	0.286	0.096
ABA ×1 vs. ×2		--	--	--	0.341	0.488

SF 800, Sumer Flavor 800; SS 5244, Summer Sweet 5244; DBT, days before transplanting.
[z]Stem height was measured from the medium surface to the shoot apex, and shoot height was measured up to the highest leaf tip by stretching the leaves.
[y]Treatments were as follows: no spray control, three timings of single spray with 1.9 mM ABA, and one treatment of double spray with 0.95 mM ABA. In all treatments, the application rate was 0.55 mg ABA per plant with the spray volume of 0.61 $L \cdot m^{-2}$ (1.1 ml/plant).
[x]Orthogonal contrasts tested two hypotheses: control vs. all ABA treatments (control vs. ABA) and all single-spray treatments vs. double-spray treatment (ABA ×1 vs. ×2).
[w]For each cultivar, least squares means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

'Summer Sweet 5244', the corresponding reductions for the 25, 18, and 11 DBT treatments were 54% (0.59 vs. 0.27 mm·cm^{-1}·d^{-1}), 40% (0.11 vs. 0.07 mm·cm^{-1}·d^{-1}), and 29% (0.07 vs. 0.05 mm·cm^{-1}·d^{-1}), respectively. These reductions were significant only for the 25 DBT treatment in both cultivars. During the subsequent measurement periods, however, the 25 DBT treatment showed higher RSER than the control by 154% in 'Summer Flavor 800' (0.14 vs. 0.35 mm·cm^{-1}·d^{-1}) and by 158% in 'Summer Sweet 5244' (0.11 vs. 0.29 mm·cm^{-1}·d^{-1}). As a result, final stem height showed no significant difference among the treatments, ranging from 3.58 to 3.96 cm in 'Summer Flavor 800' and from 3.67 to 4.22 cm in 'Summer Sweet 5244' (Table 2.6).

Table 2.7. Relative stem elongation rate (RSER) of diploid 'Sumer Flavor 800' and triploid 'Summer Sweet 5244' watermelon seedlings as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 3).

		RSER (mm·cm^{-1}·d^{-1})		
		DBT		
Cultivar	Treatment[z]	25–18	18–11	11–1
SF 800	Control	0.662 a[y]	0.139 bc	0.135
	11 DBT (1.9 mM)	--	--	0.112
	18 DBT (1.9 mM)	--	0.064 c	0.170
	25 DBT (1.9 mM)	0.348 b	0.352 a	0.128
	25 + 18 DBT (0.95 mM)	0.543 a	0.200 b	0.124
SS 5244	Control	0.591 a	0.111 bc	0.074
	11 DBT (1.9 mM)	--	--	0.053
	18 DBT (1.9 mM)	--	0.066 c	0.102
	25 DBT (1.9 mM)	0.274 c	0.287 a	0.051
	25 + 18 DBT (0.95 mM)	0.449 b	0.186 ab	0.060

SF 800, Sumer Flavor 800; SS 5244, Summer Sweet 5244; DBT, days before transplanting.
[z]Treatments are as described in Table 1.
[y]For each cultivar, least squares means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

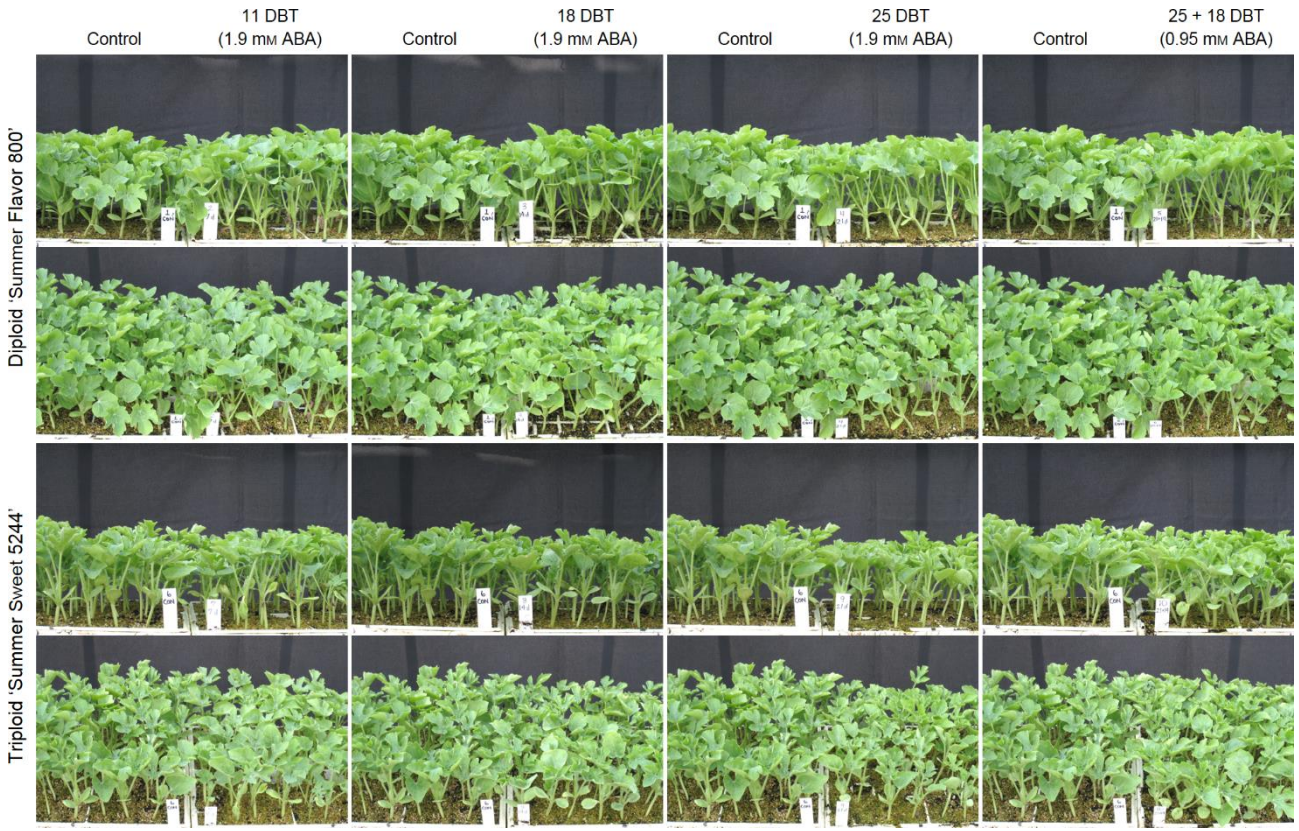

Fig. 2.16. Diploid 'Summer Flavor 800' and triploid 'Summer Sweet 5244' watermelon seedlings 1 d before transplanting (DBT) (Study 3). Treatments were as follows: no spray control, three timings of single spray with 1.9 mM ABA, and one treatment of double spray with 0.95 mM ABA. In all treatments, the application rate was 0.55 mg ABA per plant with the spray volume of 0.61 $L \cdot m^{-2}$ (1.1 ml/plant). Height and leaf area reductions by the 25 DBT treatment were readily visible in both cultivars. Cotyledon abscission was induced by the 25 + 18 DBT treatment in 'Summer Flavor 800'.

At 1 DBT, shoot height was 3–4 times higher than stem height (Table 2.6) because of petiole elongation (Fig. 2.16). The 25 DBT treatment had 14% and 13% lower shoot height than the control in 'Summer Flavor 800' (15.7 vs. 13.5 cm) and 'Summer Sweet 5244' (14.0 vs. 12.2 cm), respectively, whereas other ABA treatments were not significantly different from the control. The reductions in shoot height were highly correlated with the inhibition in petiole elongation (Fig. 2.17). Among the ABA treatments, neither multiple comparisons nor orthogonal contrasts detected a significant difference in shoot height of both cultivars. Height suppression with shortened petioles was readily visible in the 25 DBT treatment (Fig. 2.16).

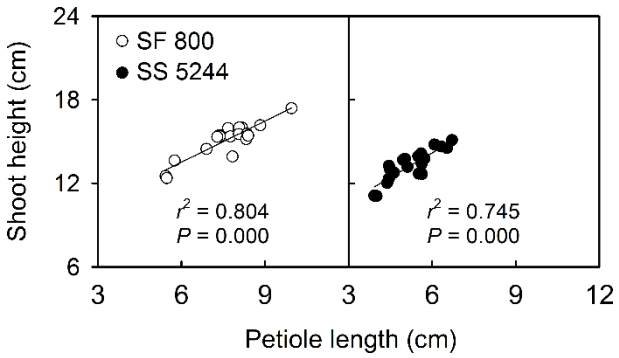

Fig. 2.17. Linear correlation between shoot height and petiole length of diploid 'Summer Flavor 800' (open symbol) and triploid 'Summer Sweet 5244' (black symbol) seedlings 1 d before transplanting (Study 3). Treatments are as described in Fig. 1.

2.3.3.2 Stem Diameter

Stem diameter at 1 DBT was smaller in the ABA treatments than in the control by 4% to 7% in 'Summer Flavor 800' (5.54 vs. 5.17 to 5.32 mm) and by 1% to 6% in 'Summer Sweet 5244' (5.16 vs. 4.87 to 5.09 mm) (Fig. 2.18). These reductions were not significant, except when all ABA treatments in 'Summer Flavor 800' were pooled (5.26 mm) by orthogonal contrasts.

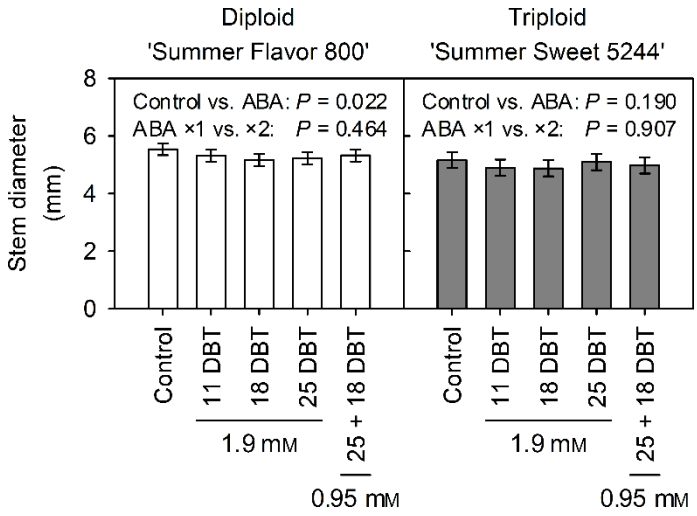

Fig. 2.18. Stem diameter of diploid 'Summer Flavor 800' (open bar) and triploid 'Summer Sweet 5244' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 3). Treatments are as described in Fig. 1. Data are least squares means ± 95% confidence intervals (n = 4). Orthogonal contrasts tested two hypotheses: control vs. all ABA treatments (control vs. ABA) and all single-spray treatments vs. double-spray treatment (ABA ×1 vs. ×2).

2.3.3.3 Leaf Growth

Cotyledon abscission was severe in the 25 + 18 DBT treatment of 'Summer Flavor 800' (Fig. 2.16), reducing cotyledon number by 46% (2.00 vs. 1.08) and area by 41% (7.55 vs. 4.42 cm^2) compared with the control (Fig. 2.19A and B). In this cultivar, orthogonal contrasts also found significant differences in two additional hypothesis tests. First, the pooled ABA treatments had smaller number (2.00 vs. 1.67) and area (7.55 vs. 6.26 cm^2) of cotyledons than the control. Second, the pooled single-spray treatments had larger number (1.86 vs. 1.08) and area (6.87 vs. 4.42 cm^2) of cotyledons than the 25 + 18 DBT treatment. In 'Summer Sweet 5244', cotyledon abscission by ABA was minimal and non-significant.

True leaves measured at 1 DBT showed similar responses to ABA in the two cultivars; none of the ABA treatments affected leaf number (Fig. 2.19A), whereas the 25 DBT treatment reduced leaf area by 17% compared with the control (40.4 vs. 33.7 cm^2 in 'Summer Flavor 800'

and 42.1 vs. 34.9 cm^2 in 'Summer Sweet 5244') (Fig. 2.19B). These leaf area reductions were readily visible (Fig. 2.16). Contrasting results in the two cultivars were also found by orthogonal contrasts. The pooled ABA treatments had smaller leaf area than the control in 'Summer Sweet 5244' (42.1 vs. 37.6 cm^2), whereas the pooled single-spray treatments had smaller leaf area than the 25 + 18 DBT treatment in 'Summer Flavor 800' (36.7 vs. 40.7 cm^2).

Leaf chlorophyll index of the youngest fully open leaf and the largest leaf was unaffected by ABA in both cultivars (data not shown). Accordingly, leaf chlorosis was not noticeable in all ABA treatments (Fig. 2.16).

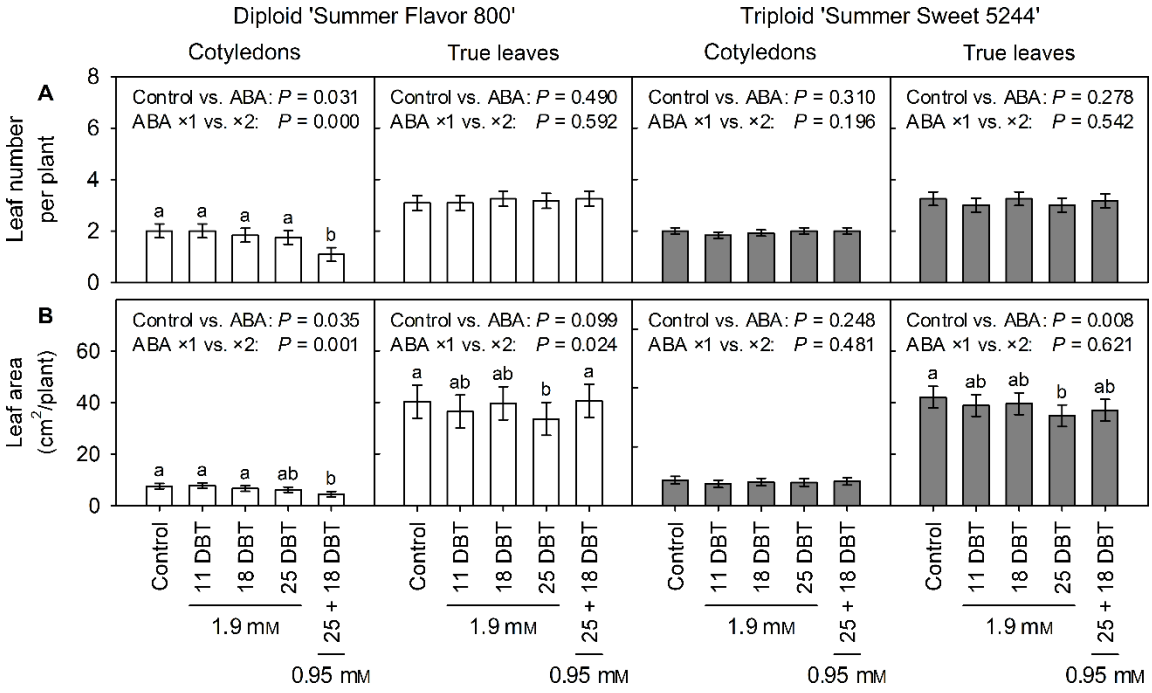

Fig. 2.19. Leaf growth of diploid 'Summer Flavor 800' (open bar) and triploid 'Summer Sweet 5244' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 3): (**A**) leaf number and (**B**) leaf area. Treatments and statistical comparisons are as described in Fig. 1 and Fig. 3, respectively. Means ± 95% confidence intervals (n = 4) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

2.3.3.4 Dry Matter Accumulation and Partitioning

Shoot dry matter accumulation at 1 DBT was inhibited by all ABA treatments in the two cultivars (Fig. 2.20A). The reductions were 17% to 21% in 'Summer Flavor 800' (391 vs. 309 to 325 mg) and 16% to 23% in 'Summer Sweet 5244' (465 vs. 357 to 388 mg), compared with the corresponding controls.

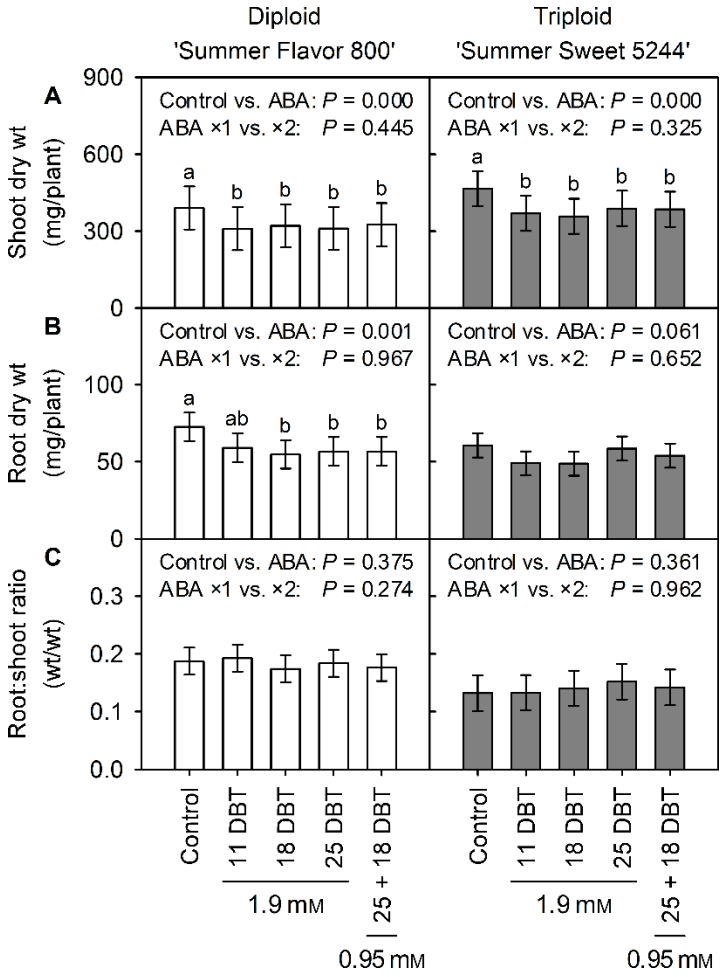

Fig. 2.20. Dry matter accumulation and partitioning of diploid 'Summer Flavor 800' (open bar) and triploid 'Summer Sweet 5244' (gray bar) seedlings 1 d before transplanting (DBT) as affected by abscisic acid (ABA) applied as a single high dose or repeated low doses (Study 3): (**A**) shoot dry weight, (**B**) root dry weight, and (**C**) root-to-shoot ratio. Treatments and statistical comparisons are as described in Fig. 1 and Fig. 3, respectively. Means ± 95% confidence intervals (n = 4) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

Root dry matter accumulation at 1 DBT was inhibited by ABA only in 'Summer Flavor 800' (Fig. 2.20B). The 11 DBT treatment (59 mg) had the smallest and non-significant inhibition, whereas other ABA treatments (55 to 57 mg) significantly reduced root dry weight by 22% to 25% compared with the control (73 mg). In this cultivar, the pooled ABA treatments (57 mg) also had significantly lower root dry weight than the control. A similar but non-significant trend for ABA treatments ($P = 0.061$) was found in 'Summer Sweet 5244'. Root-to-shoot ratio was unaffected by ABA, ranging from 0.17 to 0.19 in 'Summer Flavor 800' and from 0.13 to 0.15 in 'Summer Sweet 5244' (Fig. 2.20C).

In both cultivars, shoot dry weight was positively correlated with leaf area, whereas it had no significant correlation with shoot height and stem diameter (Fig. 2.21).

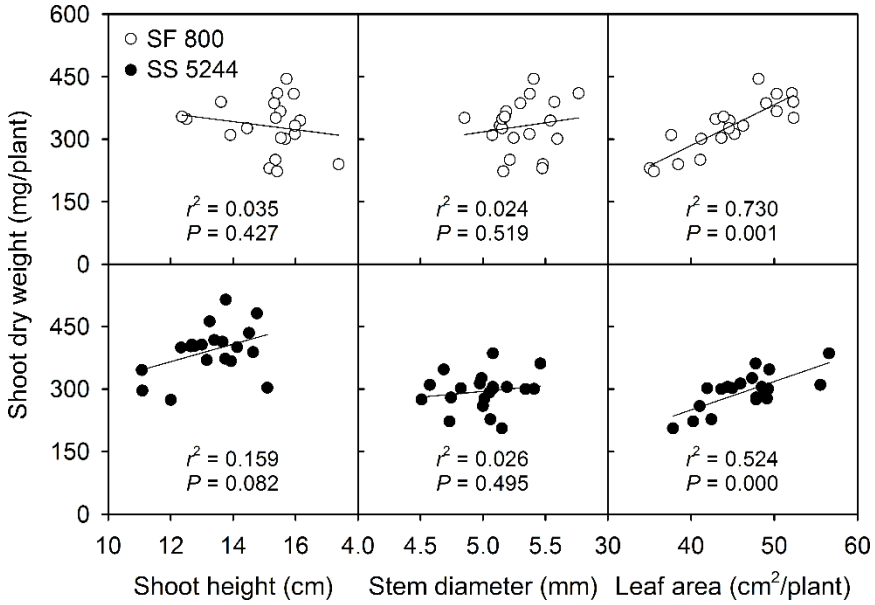

Fig. 2.21. Linear correlation between shoot dry weight and other growth variables of diploid 'Summer Flavor 800' (open symbol) and triploid 'Summer Sweet 5244' (black symbol) seedlings 1 d before transplanting (Study 3). Treatments are as described in Fig. 1.

2.3.3.5 Field Growth, Yield, and Fruit Quality

Seedling survival rate and leaf chlorophyll index showed no significant difference among the treatments (data not shown). Seedling loss was due mainly to wind damage before vine development, averaging 15% in 'Summer Flavor 800' and 11% in 'Summer Sweet 5244'. Vine length showed no significant difference among the treatments in 'Summer Flavor 800' (Table 2.8). By contrast, vine development of 'Summer Sweet 5244' was delayed in the ABA-treated plants, with vine length at 44 DAT ranging from 52% to 77% of the control. These

Table 2.8. Vine development of diploid 'Sumer Flavor 800' and triploid 'Summer Sweet 5244' watermelon as affected by abscisic acid (ABA) applied during transplant growth as a single high dose or repeated low doses (Study 3).

		Vine length (cm)		
		Time after transplanting (d)		
Cultivar	Treatment[z]	23	44	65
SF 800	Control	7.8	78	284
	11 DBT (1.9 mM)	7.5	74	304
	18 DBT (1.9 mM)	9.7	70	296
	25 DBT (1.9 mM)	6.9	74	293
	25 + 18 DBT (0.95 mM)	9.7	91	323
Orthogonal contrasts[y]			*P* value	
Control vs. ABA		0.548	0.970	0.465
ABA ×1 vs. ×2		0.133	0.136	0.371
SS 5244	Control	6.7	87	282
	11 DBT (1.9 mM)	6.0	67	260
	18 DBT (1.9 mM)	6.6	45	262
	25 DBT (1.9 mM)	5.6	65	279
	25 + 18 DBT (0.95 mM)	4.8	51	270
Orthogonal contrasts			*P* value	
Control vs. ABA		0.527	0.019	0.424
ABA ×1 vs. ×2		0.399	0.482	0.876

SF 800, Sumer Flavor 800; SS 5244, Summer Sweet 5244; DBT, days before transplanting.
[z]Treatments are as described in Table 1.
[y]Orthogonal contrasts are as described in Table 1.

reductions were significant when all ABA treatments were pooled and compared with the control (87 vs. 57 cm). At the initial stage of fruit set (65 DAT), however, the ABA-treated plants had equivalent vine development compared to the control. Yield and fruit quality variables showed no significant difference among the treatments in both cultivars (Table 2.9).

Table 2.9. Marketable yield and fruit quality of diploid 'Sumer Flavor 800' and triploid 'Summer Sweet 5244' watermelon as affected by abscisic acid (ABA) applied during transplant growth as a single high dose or repeated low doses (Study 3).

		Marketable yield			Firmness	SSC
Cultivar	Treatment[z]	(Fruit no./plant)	(kg/fruit)	(t·ha^{-1})	(N)	(°Brix)
SF 800	Control	1.25	8.55	55.8	13.0	9.71
	11 DBT (1.9 mM)	1.24	8.30	54.8	13.1	9.54
	18 DBT (1.9 mM)	1.08	8.23	50.1	13.9	10.36
	25 DBT (1.9 mM)	1.21	8.03	51.6	13.4	10.10
	25 + 18 DBT (0.95 mM)	1.14	8.50	51.3	13.0	9.69
Orthogonal contrasts[y]				*P* value		
Control vs. ABA		0.766	0.579	0.740	0.680	0.583
ABA ×1 vs. ×2		0.882	0.551	0.944	0.607	0.447
SS 5244	Control	1.02	7.57	42.2	11.0	9.78
	11 DBT (1.9 mM)	0.96	7.19	36.7	10.7	9.56
	18 DBT (1.9 mM)	1.05	6.69	37.9	10.5	9.28
	25 DBT (1.9 mM)	1.10	6.81	40.6	11.8	9.23
	25 + 18 DBT (0.95 mM)	1.00	6.94	36.1	12.5	9.86
Orthogonal contrasts				*P* value		
Control vs. ABA		0.978	0.127	0.616	0.702	0.415
ABA ×1 vs. ×2		0.870	0.912	0.779	0.174	0.183

SSC, soluble solids content; SF 800, Sumer Flavor 800; SS 5244, Summer Sweet 5244; DBT, days before transplanting.
[z]Treatments are as described in Table 1.
[y]Orthogonal contrasts are as described in Table 1.

2.3.4 Discussion

2.3.4.1 Height Control Effects of Abscisic Acid

Single spray of 1.9 mM ABA at the cotyledon stage (25 DBT) suppressed watermelon transplant height by inhibiting petiole elongation. This effect was similar in the two cultivars, diploid 'Summer Flavor 800' and triploid 'Summer Sweet 5244', with 13% to 14% reductions at the transplanting stage compared with the control. Exogenous ABA has been reported to inhibit stem elongation in many species (Biai et al., 2011; Latimer and Mitchell, 1988; Leskovar and Cantliffe, 1992; Yamazaki et al., 1995). Although the advantage of this height control effect may be limited for watermelon transplants because of their relatively short stems, our results suggest that ABA is still effective in improving transplant compactness by shortening petiole length.

2.3.4.2 Undesirable Growth Modifications by Abscisic Acid

In addition to height suppression, other growth modifications were induced by ABA. In both watermelon cultivars, all ABA treatments reduced shoot biomass compared with the control to a similar extent. The reductions ranged from 16% to 23%, which were highly associated with leaf area reductions. These results suggest that ABA inhibits leaf expansion more strongly than shoot elongation, thereby causing shoot growth reductions. Delaying transplant growth is not desirable for commercial nurseries because it prolongs the transplant production cycle and increases the cost of production.

Additional undesirable growth modifications were observed only in diploid 'Summer Flavor 800'. First, stem diameter was reduced by ABA, suggesting that ABA may weaken stem strength and thus limit the benefit of height control. This effect is a drawback to ABA treatments compared with mechanical transplant conditioning methods, which can both shorten and thicken stems by stimulating ethylene production (Garner and Björkman, 1996; Garner and Björkman,

1997; Hiraki and Ota, 1975; Latimer, 1998). Another drawback was the strong inhibition of root biomass accumulation. Large root systems are important to facilitate pulling of transplants from trays (Vavrina, 2002), whereas insufficient roots can result in severe transplant shock (Agehara and Leskovar, 2012). Furthermore, cotyledon abscission, a sign of poor transplant quality, was induced by the double-spray treatment. The ABA-induced abscission can be attributed to the stimulation of ethylene production (Gepstein and Thimann, 1981), which mediates degradation of cell wall and middle lamella by inducing the synthesis of hydrolytic enzymes (Mishra et al., 2008a; Taylor et al., 1991; Tucker et al., 1991).

In contrast to shoot height, other growth variables showed cultivar-dependent responses to ABA, with more undesirable growth inhibitions occurring in 'Summer Flavor 800' than in 'Summer Sweet 5244'. Such information is important in developing ABA application methods optimized for diploid and triploid watermelon cultivars.

2.3.4.3 Age-dependent Sensitivity to Abscisic Acid

For pepper transplants, Biai et al. (2011) suggest that ABA application should be initiated at the cotyledon stage for maximal height suppression. However, this recommendation is based solely on plant height, although other growth variables are also known to be affected by ABA (Taiz and Zeiger, 2010). In the two watermelon cultivars used in this study, age-dependent sensitivity to ABA was evident in shoot height and leaf area. In all cases, growth inhibition was maximal when 1.9 mM ABA was applied at the cotyledon stage, during which relative growth rate was most rapid. This similar age-dependent sensitivity of the two growth variables to ABA raises a dilemma in deciding the optimal application timing, because ABA can limit plant photosynthetic capacity as a trade-off for height control. However, moderately restricted leaf expansion of transplants in greenhouses may be beneficial in reducing transplant shock under

stressful field conditions, as it reduces plant water use by limiting transpirational area (Agehara and Leskovar, 2012).

2.3.4.4 Single vs. Double Application of Abscisic Acid

To avoid undesirable side effects of ABA, such as leaf chlorosis and abscission (Agehara and Leskovar, 2012; Kim and van Iersel, 2011; Waterland et al., 2010c), repeated application of low doses may be a more effective strategy than applying a single high-dose. As opposed to our assumption, cotyledon abscission was induced only by the double-spray treatment in diploid 'Summer Flavor 800', whereas other growth variables showed minimal differences between the two application strategies in both cultivars. These results suggest that to prevent cotyledon abscission, repeated application of ABA is not recommended for watermelon transplants even at low doses.

2.3.4.5 Abscisic Acid Effects Are Minimal after Transplanting

Field performance must be evaluated to justify the advantages of transplant growth modification in nurseries. Except for the relatively slow vine development, the ABA-treated plants had similar growth, yield, and fruit quality compared with the control plants. Their initial slow growth could be due simply to insufficient leaf area and root system to support new growth.

It is important to note that all transplants used in the field experiment were shipped from the nursery in trays and thus were minimally damaged. In common commercial operations, mechanical injury often occurs when transplants are pulled from trays and packed in boxes at high density for shipment (Cantliffe, 1993). Therefore, our field data may not reflect the advantage of height suppression to minimize damage during commercial shipping operations.

2.3.4.6 Practical Implications of Growth Inhibition by Abscisic Acid

Although foliar spray of ABA at the cotyledon stage can reduce watermelon transplant height, the benefit is limited by overall growth reductions, which can occur regardless of application timing. On the other hand, moderate shoot growth delay by ABA in triploid 'Summer Sweet 5244' may be of value as a growth holding strategy when transplanting is delayed because of inclement weather at the time of field establishment. Importantly, field evaluations suggest that the growth modulation by ABA is only transient with no negative impact on marketable yield and fruit quality.

2.4 Study 5: Optimizing Spray Concentration and Volume of Abscisic Acid for Height Control of Jalapeño Transplants

Abscisic acid applied as a foliar spray was evaluated for height control in jalapeño pepper seedlings. Using 3.8 mM ABA, we first compared three application timings (1–2, 3–4, and 4 leaf stages). The application at 1–2 leaf stage was most effective, reducing stem length and total leaf area by 9% and 12%, respectively, while increasing root-to-shoot ratio by 7%. Importantly, there was no negative effect on transplant appearance and yield. Using this application timing, we next compared ABA treatments in factorial combinations of three concentrations (0, 3.8, or 7.6 mM) and three spray volumes (0.2, 1, or 2 $L \cdot m^{-2}$), providing up to 4.7-mg ABA per plant. Although stem length and total leaf area decreased proportionally to the amount of ABA by up to 24% and 52%, respectively, root-to-shoot ratio was unaffected by ABA. The reduction in leaf area was due mostly to cotyledon abscission, which was significantly induced with ≥ 1.18 mg ABA per plant. Despite this negative side-effect, yield was unaffected by ABA. To determine the optimal application rate with minimum negative side-effects, an additional test was conducted using four

concentrations (0, 1.3, 2.5, or 3.8 mM) and three spray volumes (0.2, 0.4, or 0.6 $L \cdot m^{-2}$), with up to 0.71 mg ABA per plant. These rates did not induce cotyledon abscission and reduced stem length and total leaf area by up to 23% and 27%, respectively. Our results suggest that excess levels of ABA in a single foliar spray induce undesirable growth inhibitions. With optimal rates, this method provides effective height control and its extent can be easily modified by changing the concentration or spray volume.

2.4.1 Introduction

Height control of vegetable transplants is important for improving their adaptability to transplanting. Because vegetable transplants are typically grown in high-density plug trays with high competition for light, their stems are often excessively elongated and weak. Compared with stocky transplants, such transplants are more difficult to handle and are easily damaged during shipping. They are also prone to damage and skips during mechanical transplanting. As a result, their field establishment is slow and non-uniform, delaying early harvest and limiting marketable yield.

The cellular basis for stem elongation is a combination of cell division and cell elongation, both of which are known to be stimulated by gibberellins (Sachs, 1965; Taiz and Zeiger, 2010). Ethylene has antagonistic effects, such that it inhibits cell elongation and induces stem thickening (Zarembinski and Theologis, 1994). In ornamentals and flowers, several gibberellin inhibitors, such as daminozide, paclobutrazol, and uniconazole, are commercially used to produce compact plants (Gibson and Whipker, 2001; Whipker et al., 2000). However, they tend to have long-term negative effects on growth and development (Cantliffe, 1993; Latimer, 1991), and only uniconazole was recently approved for vegetable crops. Alternatively, stem elongation can be reduced by mechanical stimulation that stimulates ethylene production

(Björkman, 1999; Garner and Björkman, 1996; Garner and Björkman, 1997; Hiraki and Ota, 1975), but its commercial application is limited by a lack of automation and high labor cost (Latimer, 1998).

Abscisic acid is another plant growth regulator, which application has shown to inhibit stem elongation (Leskovar and Cantliffe, 1992; Yamazaki et al., 1995). In contrast to the gibberellin inhibitors, ABA can be rapidly inactivated by oxidation or conjugation (Davies and Jones, 1991). In jalapeño pepper (*Capsicum annuum* L.) transplants, we found that 3.8 mM ABA applied at one- to two-leaf stage reduced stem length by 9% and increased root-to-shoot ratio by 7%, with no negative effect on field growth and yield (unpublished data). The objective of this study was to optimize the application rate of ABA for the most desirable height control in jalapeño pepper transplants.

2.4.2 Materials and Methods

2.4.2.1 Plant Material and Treatments

Two experiments were conducted with 'Colima' jalapeño pepper. Seeds were sown in a polystyrene tray with 200 inverted pyramid cells each containing 23 mL of peat-lite mix on 25 Mar. 2011 and 29 Feb. 2012. In the first experiment (Study 4-1), seedlings were grown in a temperature-controlled greenhouse (15 to 30°C) at the Texas AgriLife Research and Extension Center in Uvalde, TX. At one- to two-leaf stage [28 d after seeding (DAS)], seedlings were treated with ABA (VBC-30151; Valent BioSciences, Libertyville, IL) in factorial combinations of three concentrations (0, 3.8, and 7.6 mM) and three spray volumes (0.2, 1, and 2 $L \cdot m^{-2}$), providing up to 4.7 mg ABA per plant (Table 1). In the second experiment (Study 4-2), seedlings were grown at a commercial nursery greenhouse (Speedling) located in Alamo, TX with average daily air temperature ranging from 11 to 34°C. At one- to two-leaf stage (19 DAS), seedlings

were treated with ABA in factorial combinations of four concentrations (0, 1.3, 2.5, and 3.8 mM) and three spray volumes (0, 0.2, and 0.4 $L \cdot m^{-2}$), providing up to 0.71 mg ABA per plant (Table 2). In both experiments, ABA foliar sprays were performed using a CO_2-pressured backpack sprayer (Bellspray, Inc., Opelousas, LA) between 1100 and 1200 HR. Thereafter, seedlings were grown for additional 2 weeks to reach the size for transplanting.

2.4.2.2 Transplant Growth Measurements

Stem length was measured from the medium surface to the shoot apex between 1000 and 1100 HR immediately before ABA treatment and transplanting [14 d after treatment (DAT)]. The measurements were made non-destructively on the same plants (four plants per replication), randomly selected 1 d before ABA treatment. At each measurement time, three plants per replication were randomly sampled, and roots were washed to remove the growth medium. Leaf area was measured using the LI-3100 area meter (LI-COR, Lincoln, NE). Shoots and roots were separated and dried at 65°C for 48 h to determine dry weight.

2.4.2.3 Statistical Design and Analysis

Treatments were factorial combinations of three (Study 4-1) or four (Study 4-2) ABA concentrations and three spray volumes. In both experiments, there were four replicates (trays) with up to four subsamples (plants) for each treatment arranged in a completely randomized block design. All data analyses were run using the MIXED procedure in SAS. We tested the significance of main and interaction effects using the restricted maximum likelihood method, in which ABA concentration, spray volume and the interaction were fixed factors, and replication was a random factor. Pre-treatment data were included in the fixed factors as covariates. We compared least squares means using the Tukey–Kramer test.

2.4.3 Results

2.4.3.1 Stem Elongation

In Study 4-1, stem length was significantly affected by ABA concentration × volume interaction (Fig. 2.22A). At 0 mM ABA, spray volume had no effect on stem length (5.6 to 6.0 cm). Stem length decreased with increasing ABA concentration by 11%, 16%, and 24% at 0.2, 1, and 2 L·m^{-2}, respectively. At the highest spray volume (2 L·m^{-2}), stem elongation was completely inhibited by both 3.8 and 7.6 mM ABA (< 2% from 0 to 14 DAT, data not shown).

In Study 4-2, stem length was significantly affected only by ABA concentration (Fig. 2.22B). At 0 mM ABA, stem length ranged from 5.7 to 6.1 cm (data not shown). Averaging across the spray volumes tested, stem length decreased with increasing ABA concentration by 19%.

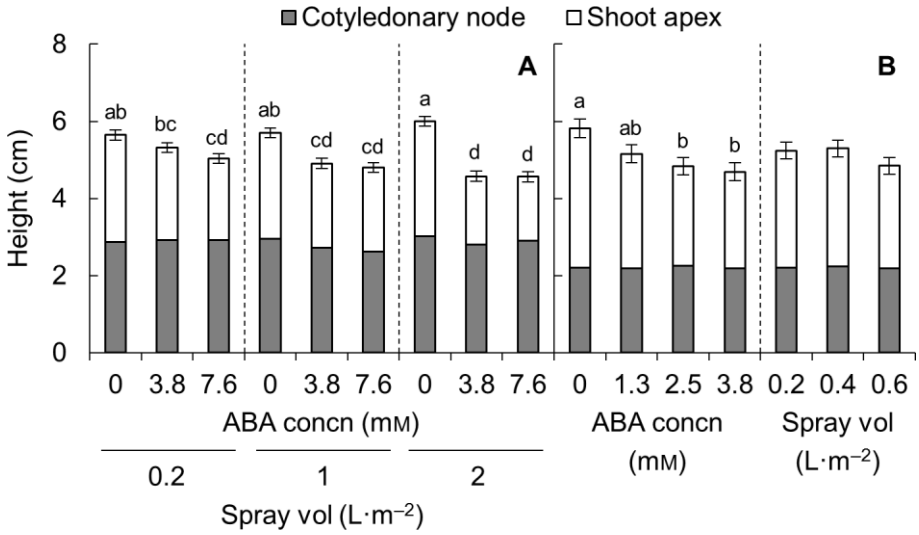

Fig. 2.22. Plant height of jalapeño pepper transplants as affected by abscisic acid (ABA) spray volume and concentration: (**A**) high-dose application in Study 4-1 and (**B**) low-dose application in Study 4-2. Seedlings were treated with ABA at 1–2 leaf stage, 14 d before the anticipated maturity date. Data are least squares means ± 95% confidence intervals. Means of stem height (from the medium surface up to the shoot apex) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Data (B) were pooled by main effects because of non-significant interaction.

2.4.3.2 Leaf Area

In Study 4-1, total leaf area (cotyledons + true leaves) was significantly affected by ABA concentration × volume interaction (Fig. 2.23A). At 0 mM ABA, spray volume had no effect on total leaf area (13.1 to 13.7 cm^2). Total leaf area decreased with increasing ABA concentration by 15%, 32%, and 52% at 0.2, 1, and 2 $L \cdot m^{-2}$, respectively. Whereas cotyledon area showed similar reductions in response to ABA, true leaf area was unaffected.

In Study 4-2, total leaf area was significantly affected only by ABA concentration (Fig. 2.23B). At 0 mM ABA, total leaf area ranged from 15.0 to 15.5 cm^2 (data not shown). Averaging across the spray volumes tested, total leaf area decreased with increasing ABA concentration by 16%. In contrast to Study 4-1, true leaf area showed similar reductions in response to ABA, but cotyledon area was unaffected.

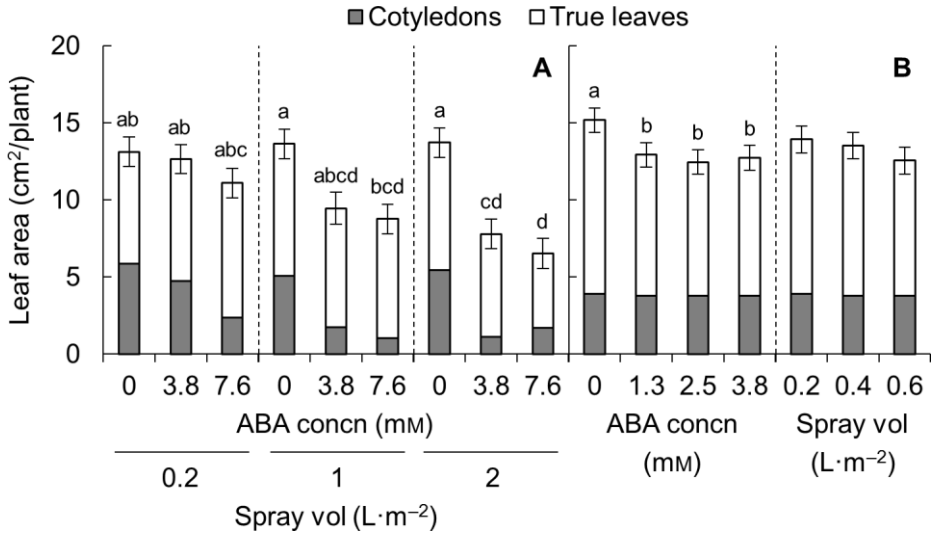

Fig. 2.23. Leaf area of jalapeño pepper transplants as affected by abscisic acid (ABA) spray volume and concentration: (**A**) high-dose application in Study 4-1 and (**B**) low-dose application in Study 4-2. Seedlings were treated with ABA at 1–2 leaf stage, 14 d before the anticipated maturity date. Data are least squares means ± 95% confidence intervals. Means of total leaf area (cotyledons + true leaves) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Data (B) were pooled by main effects because of non-significant interaction.

2.4.3.3 Dry Matter Accumulation

In Study 4-1, shoot and root dry weight were significantly affected by ABA concentration × volume interaction (Fig. 2.24A). At 0 mM ABA, spray volume had no effect on shoot dry weight (75 to 80 mg). Shoot dry weight decreased with increasing ABA concentration by 15%, 28%, and 50% at 0.2, 1, and 2 $L \cdot m^{-2}$, respectively. Similar trends were observed for

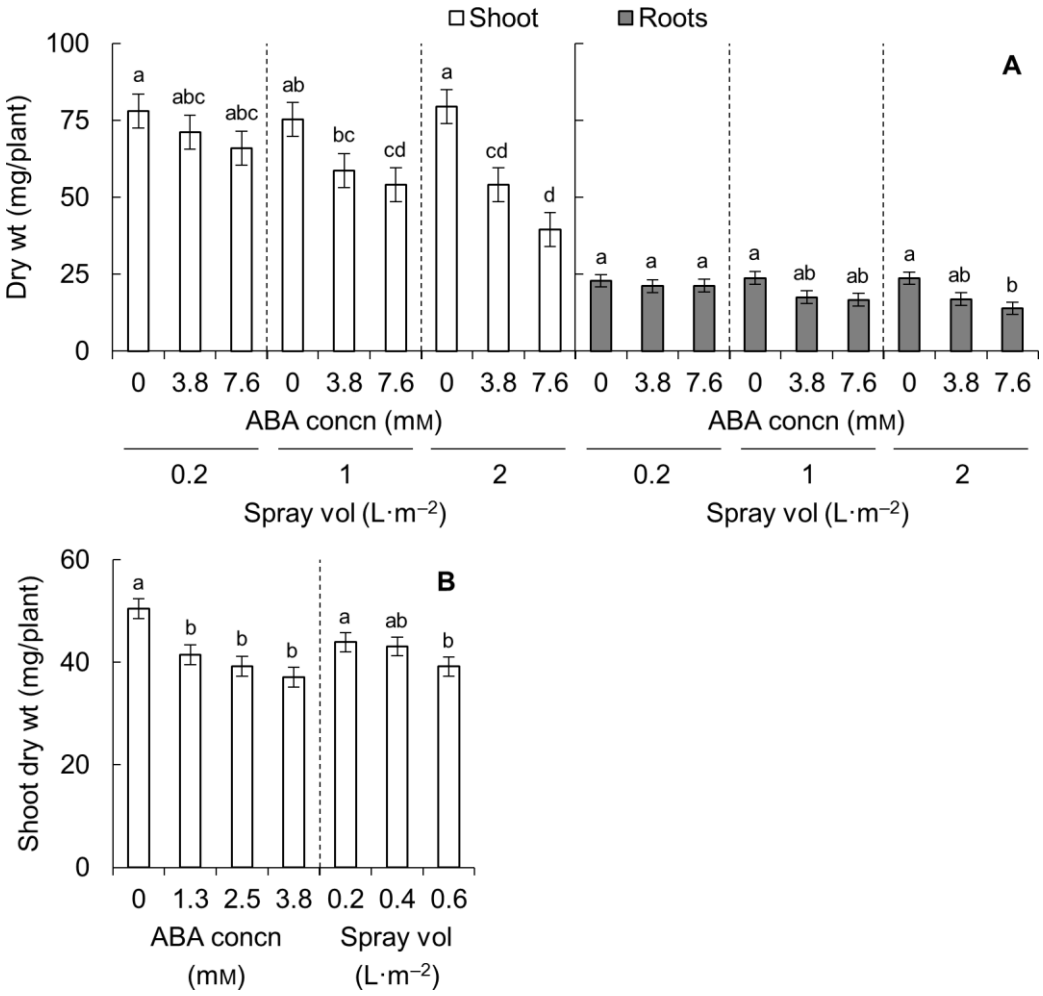

Fig. 2.24. Dry matter accumulation of jalapeño pepper transplants as affected by abscisic acid (ABA) spray volume and concentration: (**A**) high-dose application in Study 4-1 and (**B**) low-dose application in Study 4-2. Seedlings were treated with ABA at 1–2 leaf stage, 14 d before the anticipated maturity date. Data are least squares means ± 95% confidence intervals. Means with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Data (B) were pooled by main effects because of non-significant interaction.

root dry weight, which ranged from 21 to 23 mg at 0 mM ABA and decreased with increasing ABA concentration by 7%, 30%, and 41% at 0.2, 1, and 2 $L \cdot m^{-2}$, respectively.

In Study 4-2, shoot dry weight was significantly affected by ABA concentration and spray volume (Fig. 2.24B). At 0 mM ABA, shoot dry weight ranged from 49 to 52 mg (data not shown). When data were pooled by main effects, shoot dry weight decreased with increasing ABA concentration and spray volume by 26% and 11%, respectively.

2.4.4 Discussion

Foliar sprays of ABA were effective in height control of jalapeño pepper seedlings by inhibiting internode elongation mainly between the cotyledonary node and the node of first true leaf (data not shown). This effect was proportional to the application rate, with reductions in stem length ranging from 5% to 24%. The magnitude of height control is comparable to that previously reported for ABA treatments in bell pepper seedlings (Biai et al., 2011; Leskovar and Cantliffe, 1992). Similar results have been reported also for mechanical conditioning. In tomato seedlings, brushing the upper canopy 10 strokes per day reduced the final stem length by 20% (Björkman, 1998).

To produce stocky, strong transplants, it is advantageous if stems are not only short but also thick. However, stem diameter was reduced by 14% to 20% with ≥ 3.8 mM ABA at 2 $L \cdot m^{-2}$ (data not shown), suggesting that excess levels of ABA may weaken stem strength and limit the benefit of height control. This effect is contrary to mechanical stimulation, which can produce shorter and thicker stems by increasing its intensity (Garner and Björkman, 1996; Garner and Björkman, 1997).

Another drawback to ABA treatments was overall growth reductions. Application rates of ≥ 1.18 mg ABA per plant were particularly excessive, inducing severe cotyledon abscission

with reductions in total leaf area by 31% to 52%. Similar reductions were observed for shoot (22% to 50%) and root (26% to 41%) dry matter, with no significant change on their partitioning patterns. However, these growth inhibitions remained only transiently in the field and had no effect on yield and fruit quality (data not shown).

Our results suggest that, if a small to medium reduction in transplant size is acceptable, ABA can provide effective height control in a single foliar application. In contrast to mechanical stimulation, ABA is a more flexible treatment because its effectiveness can be easily modified by changing the concentration or spray volume. To avoid negative side-effects (e.g. cotyledon abscission and excessive growth reductions), ABA should not be applied in excess of 1.18 mg per plant. On the other hand, moderate growth delay by ABA may be of value as a growth retardant (to extend the marketing period of transplants), rather than as a tool for height control.

2.5 Study 5: Growth Suppression by Exogenous Abscisic Acid and Uniconazole for Prolonged Marketability of Bell Pepper Transplants under Commercial Conditions

Vegetable transplants quickly outgrow their marketability, providing limited marketing flexibility for commercial nurseries. Abscisic acid and uniconazole can suppress shoot growth by inducing stress-adaptive responses and inhibiting gibberellin biosynthesis, respectively. We evaluated their effectiveness in prolonging marketability of 'Aristotle' and 'Revolution' bell pepper transplants at commercial nursery greenhouses. Spray treatments in the first experiment were 0 and 3.8 mM ABA at 7, 5, 3, or 1 day before the anticipated maturity date (DBM), and those in the second experiment were no spray control, 3.8 mM ABA at 7, 5, 3, or 1 DBM, and 34 μM uniconazole at 4 DBM. The two experiments showed similar results with minimal cultivar-specific effects. Different growth modifications were induced by ABA and uniconazole. First,

suppression of stem elongation by ABA was reversible by 7 days after the anticipated maturity date (DAM), whereas that by uniconazole lasted for 20 days or until 16 DAM with up to 15% reductions in stem length. Second, only ABA inhibited shoot and root dry matter accumulation. These results suggest that the growth modulating effect of uniconazole is limited to height control, which is beneficial for producing compact transplants, rather than as a growth holding strategy. By contrast, overall growth suppression by ABA is desirable for prolonging transplant marketability. Importantly, the magnitude of this growth suppression was moderate (9% to 12% shoot biomass reductions at 7–8 DAM) and mostly reversible by 14–16 DAM. Furthermore, ABA inhibited root growth relatively slowly, thereby allowing sufficient root development and increasing root-to-shoot ratio at 0 DBM. Although these growth holding effects of ABA were generally maximized when it was applied at 7 or 5 DBM, leaf chlorosis and cotyledon abscission were also induced by ABA in a similar age-dependent manner. Taken together, our results suggest that ABA application immediately before the maturity stage is an effective growth holding strategy with minimal negative-side effects for bell pepper transplants.

2.5.1 Introduction

Vegetable transplants quickly outgrow their marketability in commercial nurseries. Their limited marketing flexibility is a concern especially when transplanting is delayed because of inclement weather at the time of field establishment. Overmature transplants generally have spindly stems and excessive leaf growth, whereas their root growth is limited because of the small rooting volume of high-density plug trays (Marr and Jirak, 1990; Nishizawa and Saito, 1998). Such transplants are susceptible not only to damage during shipping and transplanting (Garner and Björkman, 1996; Shaw, 1993) but also to wind lodging after transplanting (Garner and Björkman, 1999; Latimer and Mitchell, 1988). In addition, the imbalance between

transpiration demand and water uptake capacity can result in severe transplant shock and poor stand establishment (Agehara and Leskovar, 2012).

Plant growth retardants, such as daminozide, paclobutrazol, and uniconazole, are used in ornamental plant production to improve plant compactness, marketable value, and shelf life (Currey and Lopez, 2010). These chemicals limit stem elongation and overall shoot growth by inhibiting gibberellin biosynthesis (Rademacher, 2000), and their effectiveness is well documented in many ornamental species (Blanchard and Runkle, 2007; Currey et al., 2012; Gibson and Whipker, 2001; Gibson and Whipker, 2003). However, their regulations are rather restrictive for vegetable crops. At present, the only approved chemical is uniconazole registered as Sumagic for solanaceous crops including pepper, tomato, and eggplant. This product is used primarily for height control and must be applied during early development, no later than 14 d after two to four true leaf stage. How and how long uniconazole applied at late development stages affects growth and quality of vegetable transplants is unknown.

Abscisic acid is a plant hormone, which triggers adaptive growth responses to water stress (Davies and Jones, 1991). The immediate physiological response is stomatal closure, which in turn inhibits photosynthesis and transpiration-driven mass flow of nutrients (Taiz and Zeiger, 2010; Umezawa, 2011), whereas the morphological response is inhibition of leaf expansion (Bacon et al., 1998; Van Volkenburgh, 1999). Thus, the overall effect of ABA is shoot growth suppression. The potential of ABA as a growth retardant has been studied for some vegetable transplants. For example, cucumber and tomato seedlings sprayed with 0.38 or 1.89 mM ABA had reduced transpirational water loss and stem elongation during dark storage, thereby maintaining the overall quality and optimal size for transplanting (Yamazaki et al., 1995). In bell pepper, Leskovar and Cantliffe (1992) reported that the concentration effect of ABA on stem elongation was quadratic, with height suppression occurring above 10 μM. The

effectiveness of ABA is age-dependent, and growth suppression is normally maximized when ABA is applied at the cotyledon stage (Agehara and Leskovar, 2014a; Agehara and Leskovar, 2014b; Biai et al., 2011). However, these studies have not determined the duration and, more importantly, reversibility of growth suppression by ABA in overmaturing transplants. Such information is critical to evaluate the effectiveness of ABA in prolonging transplant marketability.

For vegetable transplants, growth retardants should be applied shortly before the maturity stage to suppress excessive shoot growth to a predictable and manageable extent. It is also important that this growth suppression is followed by complete recovery with no negative side effects on plant appearance. The objective of this study is to examine the magnitude, duration, and reversibility of growth suppression by ABA and uniconazole in bell pepper seedlings.

2.5.2 Materials and Methods

2.5.2.1 Plant Material and Growth Conditions

Two experiments were conducted at commercial nursery (Speedling) greenhouses located in Alamo, TX (Study 5-1) and Ruskin, FL (Study 5-2) from Aug. to Oct. 2009. At each location, seeds of two bell pepper cultivars, ‘Aristotle’ (Seminis Vegetable Seeds, St. Louis, MO) and ‘Revolution’ (Harris Moran Seed Company, Modesto, CA), were sown in a polystyrene tray with 200 inverted pyramid cells each containing 23 mL of peat-lite mix (Speedling Peat-lite; Speedling). Seedlings were grown under commercial conditions throughout the two experiments.

2.5.2.2 Abscisic Acid Treatments

In Study 5-1, treatments were factorial combinations of two ABA concentrations [0 and 3.8 mM (1000 mg·L^{-1})] and four application timings (7, 5, 3, and 1 DBM). In Study 5-2, there were six spray treatments: no spray control, four application timings of 3.8 mM ABA (7, 5, 3, and 1 DBM), and one treatment of 34 μM (10 mg·L^{-1}) uniconazole applied at 4 DBM. The maturity date was when seedlings were anticipated to become optimal for shipping and transplanting according to the commercial nursery.

The formulation of ABA stock solution was VBC-30151 containing 10% of S-ABA, a naturally occurring active form in plants. Uniconazole was formulated as Sumagic. Test solutions were prepared immediately before each treatment by diluting the stock solutions with irrigation water at the nursery. All test solutions including the control were mixed with a non-ionic surfactant (CapSil; Aquatrols) at 0.05% (v/v), which showed no significant effect on transplant growth in our preliminary experiment.

A CO_2-pressurized backpack sprayer (Model T; Bellspray) was used to spray the test solutions evenly over the seedlings between 1000 and 1100 HR. The sprayer was equipped with three flat-fan nozzles (TP8002VS; TeeJet Technologies) and a CO_2 cylinder with pressure maintained at 276 kPa. Spray volume was 0.61 L·m^{-2} (0.71 ml/plant), which wetted the leaves thoroughly to the dripping point. The spray concentration and volume were determined based on manufacturer recommendations.

2.5.2.3 Transplant Growth Measurements

In Study 5-1, stem height, cotyledon number, and leaf chlorophyll index were measured non-destructively at 8, 6, 4, 2 and 0 DBM and 7 and 14 DAM, whereas shoot and root dry weight were measured destructively at 8 and 0 DBM and 7 and 14 DAM. Five plants per

replication were randomly selected before the first measurement. All non-destructive measurements were made repeatedly on the selected plants between 0800 and 1000 HR on each measurement day. Stem height (cm) was measured from the medium surface to the shoot apex. Relative stem elongation rate (RSER, $mm \cdot cm^{-1} \cdot d^{-1}$) was calculated as follows:

$$RSER = (\ln H_2 - \ln H_1)/(t_2 - t_1) \times 10$$

where $\ln H_1$ and $\ln H_2$ are the natural logarithm of stem height at time one, t_1, and time two, t_2, respectively.

Leaf chlorophyll index was measured using a chlorophyll meter (SPAD-502; Konica Minolta Sensing) on the youngest fully open leaf and the largest leaf. Two readings were taken per leaf on a leaf lamina between major leaf veins. At each measurement time, three plants per replication were randomly sampled, and roots were washed to remove the growth medium. Shoots and roots were separated and dried at 65°C for 72 h to determine dry weight.

In Study 5-2, stem height was measured non-destructively at 8, 6, 4, 2 and 0 DBM and 8, 16, and 29 DAM, whereas shoot and root dry weight were measured destructively at 8 and 0 DBM and 8 and 16 DAM. These measurements were made using the method described for Study 5-1.

2.5.2.4 Statistical Design and Analysis

In Study 5-1, there were four replicates for each treatment arranged in a split-plot design with application timing as the main plot and ABA concentration as the subplot. One half of each seedling tray was sprayed with the control solution, and the other half was sprayed with 3.8 mM ABA solution. In Study 5-2, there were four replicates for each treatment arranged in a split-plot design with cultivar as the main plot and spray treatment as the subplot. Each treatment of spray was assigned randomly to an individual tray.

All data analyses were run in SAS, and *P* values less than 0.05 were considered statistically significant. In both experiments, treatment effects were tested using the restricted maximum likelihood method with the DDFM=KR option in the MIXED procedure. Pre-treatment data were included as covariates. Multiple comparisons of least squares means were performed by the Tukey–Kramer test in the MIXED procedure. When heteroscedasticity was indicated by a likelihood ratio test, the MIXED procedure was run with the GROUP option in the REPEATED statement.

In Study 5-2, two specific hypotheses were also tested by orthogonal contrasts in the MIXED procedure. First, we hypothesized that all ABA treatments have equivalent growth modulating effects, thereby comparing the control with the pooled ABA treatments. Second, we hypothesized that growth modulation by ABA is different from that by uniconazole, thereby comparing the pooled ABA treatments with the uniconazole treatment.

2.5.3 Results

2.5.3.1 Abscisic Aid Effects (Study 5-1)

In both ‘Aristotle’ and ‘Revolution’, stem elongation was similar at all application timings of the control (water + surfactant), remaining constant from 8 to 0 DBM but gradually slowing down thereafter (Fig. 2.25). This growth pattern was reflected in RSER, which decreased steadily during the experiment in the control (Table 2.10). Stem elongation was inhibited by ABA similarly in the two cultivars (Fig. 2.25). Except when ABA was applied at 1 DBM, the ABA-induced growth inhibition peaked 3 d after treatment, reducing stem height by 7% to 9% (0.8–1.0 cm) in ‘Aristotle’ and by 7% to 10% (0.8–1.1 cm) in ‘Revolution’. The magnitude of height suppression remained the same until 0 DBM in the 5 and 3 DBM treatments [7% to 10% (0.8–1.1 cm) in ‘Aristotle’ and 7% to 8% (0.8–1.0 cm) in ‘Revolution’], whereas it

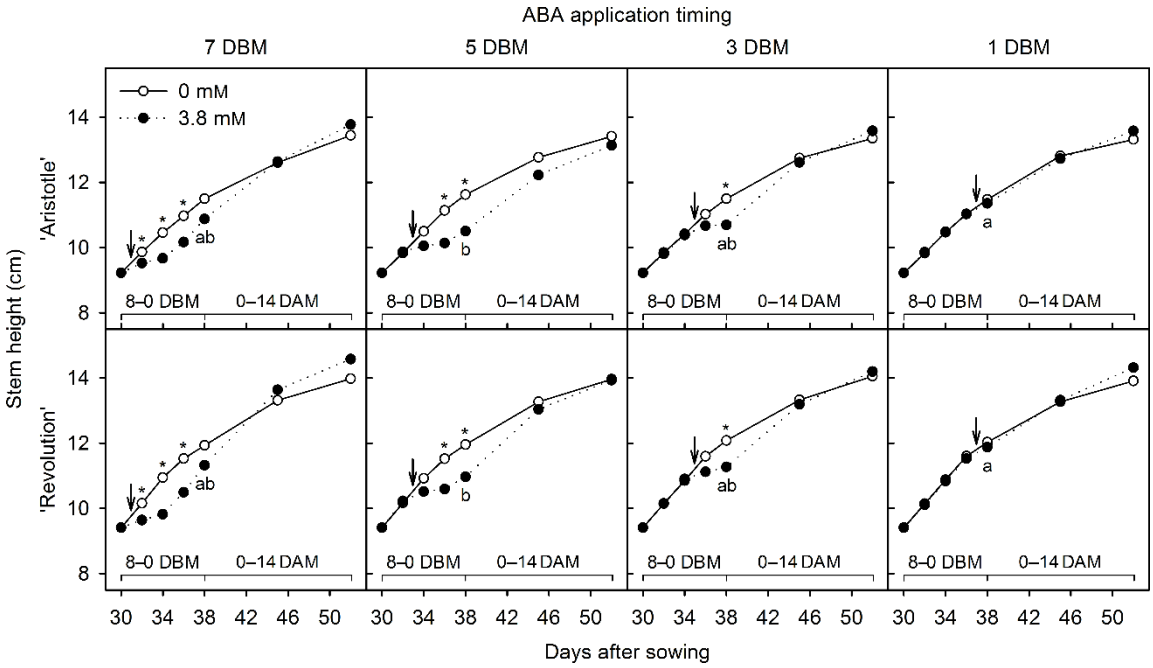

Fig. 2.25. Stem elongation rate of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by application timing of abscisic acid (ABA) (Study 5-1). Arrows indicate ABA spray application events. Data points are means ± 95% confidence intervals (n = 4). Asterisks indicate significant effects of ABA for each application timing (Tukey–Kramer test, $P < 0.05$). Data points of the 0.38 mM ABA treatment (means, n = 4) with the same letter are not significantly different across application timings (Tukey–Kramer test, $P < 0.05$). DAM = days after the anticipated maturity date. DBM = days before the anticipated maturity date.

became smaller and non-significant in the 7 DBM treatment. Correspondingly, RSER from 8 to 0 DBM was significantly reduced by ABA only when it was applied at 5 or 3 DBM (Table 2.10). Among the ABA treatments, stem height showed no significant difference at 0 DBM, except that it was 7% shorter in the 5 DBM treatment than in the 1 DBM treatment (10.5 vs. 11.4 cm in 'Aristotle' and 11.0 vs. 11.9 cm in 'Revolution') (Fig. 2.25). Thereafter, all ABA treatments had higher RSER than the corresponding controls (Table 2.10), and their height control effects became non-significant (Fig. 2.25).

Dry matter accumulation and partitioning data discussed below were pooled by ABA concentration, which was the only significant effect during the experiment (Fig. 2.26A–C).

Table 2.10. Relative stem elongation rate (RSER) of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by application timing of abscisic acid (ABA) (Study 5-1).

	Application	ABA concn	RSER ($mm \cdot cm^{-1} \cdot d^{-1}$)		
Cultivar	timing	(mM)	8–0 DBM	0–7 DAM	7–14 DAM
Aristotle	7 DBM	0.0	0.278 a[z]	0.137 b	0.088 abcd
		3.8	0.209 abc	0.228 a	0.119 a
	5 DBM	0.0	0.288 a	0.132 b	0.073 bcd
		3.8	0.159 c	0.220 a	0.104 ab
	3 DBM	0.0	0.275 a	0.148 b	0.066 cd
		3.8	0.185 bc	0.232 a	0.108 a
	1 DBM	0.0	0.269 ab	0.150 b	0.061 d
		3.8	0.261 ab	0.160 b	0.094 abc
Revolution	7 DBM	0.0	0.297 a	0.159 b	0.064 d
		3.8	0.234 ab	0.270 a	0.099 abc
	5 DBM	0.0	0.301 a	0.150 b	0.075 bcd
		3.8	0.187 b	0.248 a	0.093 abc
	3 DBM	0.0	0.305 a	0.140 b	0.071 cd
		3.8	0.225 ab	0.224 a	0.104 ab
	1 DBM	0.0	0.310 a	0.142 b	0.068 cd
		3.8	0.301 a	0.163 b	0.112 a

DAM, days after the anticipated maturity date; DBM, days before the anticipated maturity date

[z]For each cultivar, means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

Shoot dry matter accumulation was inhibited by ABA similarly in the two cultivars (Fig. 2.26A). The magnitude of growth inhibition became gradually smaller; shoot dry weight reductions by ABA in 'Aristotle' were 15% at 0 DAM (205 vs. 174 mg), 9% at 7 DAM (245 vs. 222 mg), and 2% at 14 DAM (301 vs. 295 mg), and those in 'Revolution' were 14% at 0 DAM (219 vs. 187 mg), 10% at 7 DAM (255 vs. 229 mg), and 4% at 14 DAM (290 vs. 277 mg). These reductions were statistically significant at 0 and 7 DAM in both cultivars. The partitioning of dry matter between leaves and stems was unaffected by ABA throughout the experiment (data not shown). Root dry matter accumulation was significantly affected by ABA only in 'Revolution' (Fig.

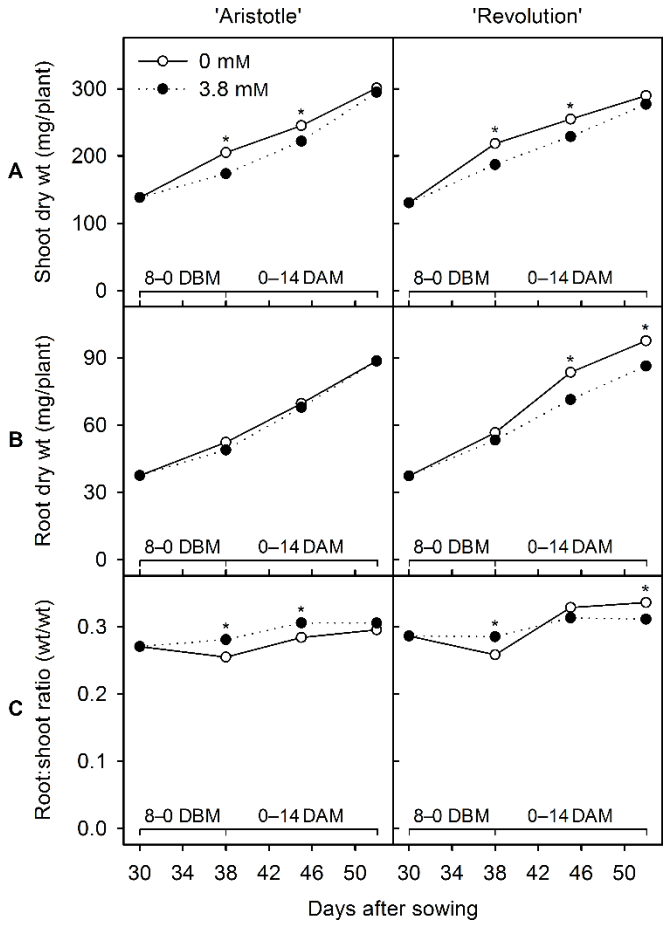

Fig. 2.26. Dry matter accumulation and partitioning of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by abscisic acid (ABA) (Study 5-1): (**A**) shoot dry weight, (**B**) root dry weight, and (**C**) root-to-shoot ratio. Treatments are same as in Fig. 2.25. Because application timing and ABA concentration × application timing effects were non-significant, data were pooled by ABA concentration. Data points are group means ± 95% confidence intervals (n = 4). Asterisks indicate significant effects of ABA (Tukey–Kramer test, $P < 0.05$).

2.26B). In this cultivar, the ABA treatment reduced root dry weight by 15% at 7 DAM (83 vs. 71 mg) and by 12% at 14 DAM (98 vs. 86 mg). Consequently, the partitioning of shoot and root dry matter was affected by ABA differently in the two cultivars (Fig. 2.26C). Although both cultivars significantly increased root-to-shoot by ABA at 0 DAM (0.255 vs. 0.281 in 'Aristotle' and 0.258 vs. 0.285 in 'Revolution'), only 'Aristotle' maintained the significant increase until 7

DAM (0.284 vs. 0.306). At 14 DAM, root-to-shoot ratio of 'Aristotle' showed no significant difference, whereas that of 'Revolution' was significantly reduced by ABA (0.336 vs. 0.311).

Leaf chlorophyll index in the control was relatively constant from 8 to 0 DBM but gradually decreased thereafter (Fig. 2.27). Leaf chlorosis, as indicated by reductions in chlorophyll index, was induced by ABA applied at 7 DBM in both cultivars. Compared with the control, chorophyll reductions by this ABA treatment in 'Aristotle' were 11% at 6 DBM (38.8 vs. 34.7) and 12% at 4 DBM (38.5 vs. 33.7), whereas those in 'Revolution' were 8% at 3 DBM (38.1 vs. 34.8). In both cultivars, the ABA-induced leaf chlorosis was corrected by 2 DBM, with chlorophyll index recovering to the control level.

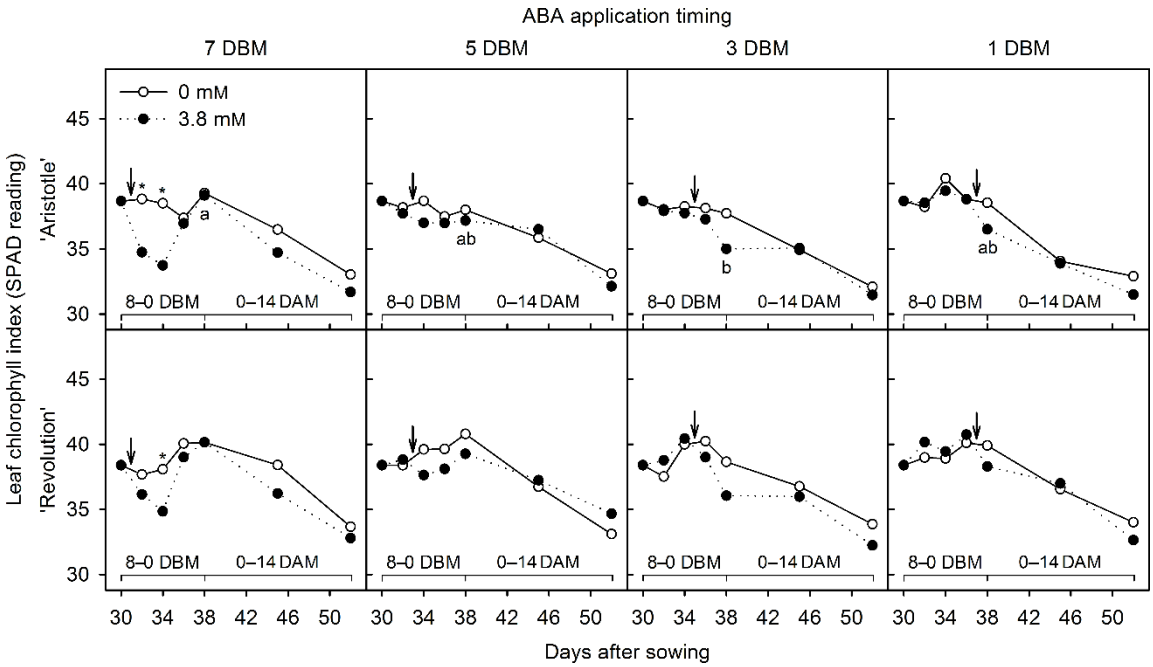

Fig. 2.27. Leaf chlorophyll index of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by application timing of abscisic acid (ABA) (Study 5-1). Arrows indicate ABA spray application events. Data points are means ± 95% confidence intervals (n = 4). Asterisks indicate significant effects of ABA for each application timing (Tukey–Kramer test, $P < 0.05$). Data points of the 0.38 mM ABA treatment with the same letter are not significantly different across application timings (Tukey–Kramer test, $P < 0.05$). DAM = days after the anticipated maturity date. DBM = days before the anticipated maturity date.

Cotyledon abscission in the control occurred after 0 DAM and was complete at 14 DAM (Fig. 2.28). In both cultivars, cotyledon abscission was accelerated by ABA, except when ABA applied at 1 DBM. In the 7 and 5 DBM treatments, the stimulation of abscission was pronounced, with at least one cotyledon abscising at 0 DAM. No abscission was observed for true leaves.

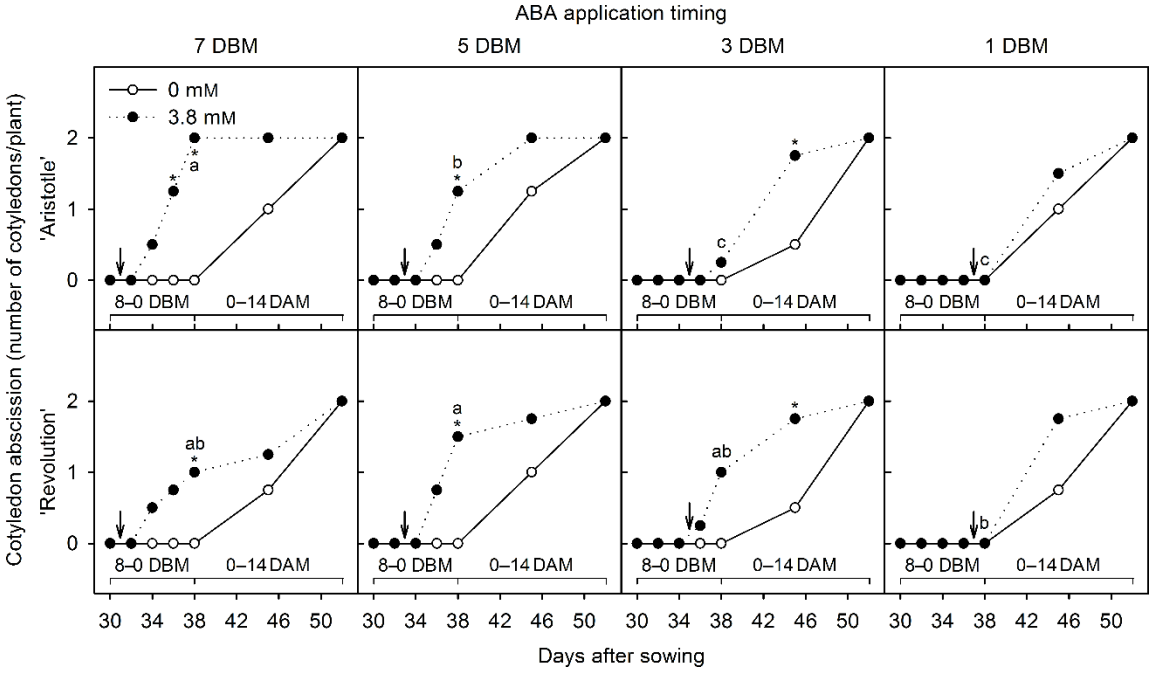

Fig. 2.28. Cotyledon abscission of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by application timing of abscisic acid (ABA) (Study 5-1). Arrows indicate ABA spray application events. Data points are means ± 95% confidence intervals (n = 4). Asterisks indicate significant effects of ABA for each application timing (Tukey–Kramer test, $P < 0.05$). Data points of the 0.38 mM ABA treatment with the same letter are not significantly different across application timings (Tukey–Kramer test, $P < 0.05$). DAM = days after the anticipated maturity date. DBM = days before the anticipated maturity date.

2.5.3.2 Abscisic Acid and Uniconazole Effects (Study 5-2)

All data discussed below were pooled by each main effect, because they were not significantly affected by the cultivar × spray treatment interaction throughout the experiment. In

Table 2.11. Stem height and relative stem elongation rate (RSER) of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by abscisic acid (ABA) and uniconazole (Study 5-2).

	Stem height (cm)					RSER ($mm \cdot cm^{-1} \cdot d^{-1}$)			
	DBM		DAM			DBM	DAM		
Treatment	8	0	8	16	29	8–0	0–8	8–16	16–29
Cultivar									
Aristotle	7.6 b[x]	9.7 b	11.4 b	12.0 b	13.5	0.208	0.223 a	0.109	0.062
Revolution	9.2 a	10.1 a	12.0 a	13.4 a	14.3	0.217	0.194 b	0.104	0.044
Spray treatment[z]									
Control	8.4	10.2 a	11.8 a	12.9 a	13.7	0.240 ab	0.167 c	0.102 b	0.045
ABA (7 DBM)	--	9.5 b	12.1 a	13.5 a	14.5	0.172 c	0.326 a	0.159 a	0.059
ABA (5 DBM)	--	9.8 ab	11.8 a	13.0 a	13.7	0.193 bc	0.242 b	0.123 ab	0.044
ABA (3 DBM)	--	10.1 a	12.1 a	13.0 a	13.8	0.260 a	0.247 b	0.114 ab	0.046
ABA (1 DBM)	--	10.0 ab	12.0 a	13.1 a	13.8	0.218 abc	0.235 b	0.103 b	0.043
Uniconazole (4 DBM)	--	10.0 ab	10.3 b	11.0 b	14.0	0.193 bc	0.032 d	0.039 c	0.081
Orthogonal contrasts[y]	*P* value								
Control vs. ABA	--	0.006	0.176	0.195	0.830	0.074	0.000	0.085	0.892
ABA vs. uniconazole	--	0.390	0.000	0.000	0.985	0.273	0.000	0.000	0.118

DAM, days after the anticipated maturity date; DBM, days before the anticipated maturity date.
[z]Spray treatments are as follows: no spray control, four application timings of 3.8 mM ABA, and one treatment of 34 μM uniconazole applied at 4 DBM
[y]Orthogonal contrasts tested two hypotheses: control vs. all ABA treatments (control vs. ABA) and uniconazole vs. all ABA treatments (uniconazole vs. ABA).
[x]For each main effect, means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

terms of the cultivar effect, similar trends were found as in Study 5-1, with ‘Revolution’ showing higher stem height and greater shoot and root dry weight than ‘Aristotle’ in most measurements (Tables 2.11 and 2.12).

Stem elongation was transiently inhibited by ABA (Table 2.11). The maximum inhibition occurred when ABA was applied at 7 DBM, reducing stem height at 0 DBM by 7% (10.2 vs. 9.5 cm) and RSER from 8 to 0 DBM by 28% (0.240 vs. 0.172 $mm \cdot cm^{-1} \cdot d^{-1}$) compared with the control. Although other ABA treatments were non-significant by multiple comparisons, the pooled ABA treatments also had significantly lower stem height than the control (10.2 vs. 9.8 cm). From 0 to 8 DAM, however, all ABA treatments had 41% to 96% higher RSER than the control (0.167 vs. 0.235–0.326 $mm \cdot cm^{-1} \cdot d^{-1}$), and the height control effect of ABA became non-significant after 0 DBM. In contrast to ABA, uniconazole induced a long-term inhibition of stem elongation. Compared with the control, the uniconazole treatment reduced stem height by 12% at 8 DAM (11.8 vs. 10.3 cm) and by 15% at 16 DAM (12.9 vs. 11.0 cm), while reducing RSER by 81% from 0 to 8 DAM (0.167 vs. 0.032 $mm \cdot cm^{-1} \cdot d^{-1}$) and by 62% from 8 to 16 DAM (0.102 vs. 0.039 $mm \cdot cm^{-1} \cdot d^{-1}$). The uniconazole treatment also showed a similar magnitude of height suppression compared with the ABA treatments. At 29 DAM, the height control effect of uniconazole became non-significant.

Shoot dry matter accumulation was transiently inhibited by ABA (Table 2.12). Compared with the control, the 7 and 5 DBM treatments reduced shoot dry weight by 22% to 24% at 0 DBM (167 vs. 127–130 mg) and by 16% to 17% at 8 DAM (245 vs. 203–205 mg). Although the 3 DBM treatment was non-significant by multiple comparisons, the pooled ABA treatments (137 mg at 0 DBM and 216 mg at 8 DAM) also had significantly lower root dry weight than the control during the same measurement period. At 16 DAM, however, these reductions became smaller and non-significant. Similar differences were observed when the

Table 2.12. Dry matter accumulation and partitioning of 'Aristotle' and 'Revolution' bell pepper seedlings as affected by abscisic acid (ABA) and uniconazole (Study 5-2).

	Shoot dry wt (mg)				Root dry wt (mg)				Root:shoot ratio (wt/wt)			
	DBM	DAM			DBM	DAM			DBM	DAM		
Treatment[z]	8	0	8	16	8	0	8	16	8	0	8	16
Cultivar												
Aristotle	92 b[x]	141	208 b	290	37 b	49	72	116	0.403	0.333	0.339	0.425
Revolution	117 a	155	235 a	299	46 a	45	76	126	0.394	0.314	0.331	0.400
Plant growth regulator												
Control	105	167 a	245 a	304	41	49	85 a	123	0.398	0.297 b	0.341	0.404
ABA (7 DBM)	--	130 b	205 b	284	--	43	66 b	108	--	0.324 ab	0.321	0.391
ABA (5 DBM)	--	127 b	203 b	282	--	44	66 b	117	--	0.357 a	0.338	0.415
ABA (3 DBM)	--	153 ab	225 ab	321	--	47	74 ab	124	--	0.307 ab	0.328	0.405
ABA (1 DBM)	--	140 b	229 ab	295	--	47	72 ab	119	--	0.341 ab	0.319	0.397
Uniconazole (4 DBM)	--	170 a	223 ab	282	--	51	83 a	133	--	0.315 ab	0.364	0.462
Orthogonal contrasts[y]	*P* value											
Control vs. ABA	--	0.000	0.001	0.454	--	0.112	0.000	0.357	--	0.015	0.341	0.923
Uniconazole vs. ABA	--	0.000	0.428	0.287	--	0.010	0.001	0.031	--	0.248	0.020	0.020

DAM, days after the anticipated maturity date; DBT, days before the anticipated maturity date.

[z]Treatments are as described in Table 2.11.

[y]Orthogonal contrasts tested two hypotheses: control vs. all ABA treatments (control vs. ABA) and uniconazole vs. all ABA treatments (uniconazole vs. ABA).

[x]For each main effect, means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

ABA treatments were compared with the uniconazole treatment. The partitioning of dry matter between leaves and stems was similar in all treatments throughout the experiment (data not shown).

The inhibitory effect of ABA on root growth was initially small, with no significant difference in root dry weight among the control and ABA treatments at 0 DBM (Table 2.12). However, root growth subsequently showed a similar response to ABA as shoot growth. At 8 DAM, the 7 and 5 DBM treatments reduced root dry weight by 22% compared with the control (85 vs. 66 mg). Although other ABA treatments were non-significant by multiple comparisons, the pooled ABA treatments also had significantly lower root dry weight than the control (85 vs. 69 mg). These reductions were non-significant at 16 DAM. By contrast, the pooled ABA treatments had significantly lower root dry weight than the uniconazole treatment throughout the experiment.

Dry matter partitioning in roots increased in response to ABA at 0 DBM because of the relatively stronger inhibition in shoot growth (Table 2.12). This effect was maximum in the 5 DBM treatment, with a 20% increase in root-to-shoot ratio compared with the control (0.297 vs. 0.357). Although other ABA treatments were non-significant by multiple comparisons, the pooled ABA treatments (0.332) also had significantly higher root-to-shoot ratio than the control. After 0 DAM, root-to-shoot ratio showed no significant difference among the control and ABA treatments. The uniconazole treatment showed no significant difference compared with the control, but it had significantly higher root-to-shoot ratio than the pooled ABA treatments at 8 and 16 DAM (0.327 vs. 0.364 and 0.402 vs. 0.462, respectively).

Slight yellowing of cotyledons and matured leaves occurred 1–2 d after ABA application (data not shown). The ABA-induced leaf chlorosis was readily visible in the 7 and 5 DBM

treatments at 0 DBM, but it became unnoticeable by 8 DAM. Cotyledon abscission was significantly induced by neither ABA nor uniconazole (data not shown).

2.5.4 Discussion

2.5.4.1 Different Growth Modulations by Abscisic Acid and Uniconazole

Spray applications of 3.8 mM ABA between 7 and 1 DBM and 34 μM uniconazole at 4 DBM were both effective in suppressing excessive growth of bell pepper transplants, but they induced different morphological changes. First, suppression of stem elongation by ABA was reversible by 7 DAM, whereas that by uniconazole lasted for 20 d or until 16 DAM with up to 15% reductions in stem length. Second, shoot and root dry matter accumulation was inhibited only by ABA. Consequently, the main effect of ABA was overall growth delay, whereas uniconazole produced more compact transplants without affecting leaf and root growth. These trends had minimal variations between the two cultivars tested.

To prolong the marketable period of vegetable transplants, overall growth delay must be induced shortly before the anticipated maturity stage to a predictable and manageable extent, followed by complete recovery. In this study, ABA rapidly inhibited shoot growth, reducing shoot biomass at the maturity stage even when it was applied at 1 DBM. By contrast, ABA inhibited root growth with a time lag of over a week, reducing root biomass beginning 7 DAM. The resulting increase in root-to-shoot ratio at the maturity stage is a preferable characteristic of hardened transplants (Vavrina, 2002). The relatively slow inhibition of root growth is also advantageous, because it ensures sufficient root development by the time of transplanting, which is necessary not only to minimize transplant shock (Agehara and Leskovar, 2012) but also to facilitate pulling of transplants from trays (Vavrina, 2002). It is reported that ABA can restrict plant growth directly by inhibiting leaf expansion (Alves and Setter, 2000; Bacon et al., 1998;

He and Cramer, 1996), or indirectly by inducing stomatal closure, which in turn inhibits photosynthesis and limits the supply of assimilates for dry matter production (Amthor, 2007; Lawlor, 2002). Importantly, the observed ABA-induced growth reductions were moderate and reversible mostly by 14 DAM.

In contrast to ABA, uniconazole had a long-term inhibitory effect on stem elongation, which took more than 30 d to be reversible. Similar long-term height control effects of uniconazole are reported in many ornamental species (Blanchard and Runkle, 2007; Currey et al., 2012; Gibson and Whipker, 2001; Gibson and Whipker, 2003). Uniconazole inhibits the synthesis of gibberellins, which are involved in both cell division and expansion (Rademacher, 2000). This mode of action appears to be more effective in height suppression than the ABA-induced growth inhibition described above. On the other hand, uniconazole had no significant effect on shoot and root dry matter accumulation. This observation is interesting because gibberellin synthesis is particularly active in young developing leaves and gibberellins stimulate cell expansion (Hedden and Kamiya, 1997; Van Volkenburgh, 1999). A possible explanation may be that stem elongation is simply more sensitive to gibberellins than leaf expansion. In fact, the most pronounced effect of exogenous gibberellins is often the stimulation of internode elongation (Taiz and Zeiger, 2010). The tissue-specific growth inhibition by uniconazole may be more beneficial for height control of vegetable transplants than as a growth holding strategy.

2.5.4.2 Short-term and Reversible Growth Inhibition by Abscisic Acid

The reversible ABA-induced growth inhibition was demonstrated by the temporal response of RSER to ABA, with a rapid decrease continuing for 3 d followed by a dramatic increase. Furthermore, both stem height and shoot biomass showed complete recovery to the control level by 7 and 14 DAM, respectively. The reversible effect of ABA has also been

observed in our previous studies. With a similar experiment setup, we have examined growth responses of pepper (bell 'Excursion II' and jalapeño 'Colima') and watermelon (diploid 'Summer Flavor 800' and triploid 'Summer Sweet 5244') seedlings to 3.8 and 1.9 mM ABA applied 25–8 d before transplanting, respectively (Agehara and Leskovar, 2014a; Agehara and Leskovar, 2014b). In bell pepper, the ABA-induced biomass reductions became non-significant at the transplanting stage. All other growth modifications were reversible upon transplanting. These transient effects of ABA are due likely to oxidation or conjugation that rapidly inactivates ABA in plant tissue (Davies and Jones, 1991). Conversely, synthetic ABA analogs are known to have long-term effects because of their high chemical stability (Abrams et al., 1997). For example, growth inhibition in tomato seedlings by 50–100 μM ABA analogs (8′-metylene ABA methyl ester and 8′-acetylene ABA methyl ester) was strong and not recovered at the end of a 9-d evaluation period, with up to a 33% reduction in shoot biomass compared with the control (Sharma et al., 2006b). In field trials with tomato and pumpkin, pre-transplanting application of 8′-acetylene ABA methyl ester at 100 μM reduced transplant shock but slowed subsequent growth, resulting in limited fruit set (Sharma et al., 2006a). Therefore, as a growth retardant, the easily degradable natural ABA appears to be more suitable than its analogs and uniconazole.

2.5.4.3 Age-dependent Effects of Abscisic Acid

Although the growth holding effect of ABA was generally maximized when it was applied at 7 or 5 DBM, undesirable growth modifications were also induced by ABA in a similar age-dependent manner. First, leaf chlorosis was induced only by ABA applied at 7 DBM, although it was reversible by the maturity stage. Second, except when ABA was applied at 1 DBM, cotyledon senescence was accelerated by ABA, with severe abscission occurring at the maturity stage. Leaf chlorosis and abscission are often reported as negative side effects of ABA

application in various crops (Agehara and Leskovar, 2012; Kim and van Iersel, 2011; Waterland et al., 2010c), which can be mediated by gene expression of hydrolytic enzymes involved in chlorophyll breakdown and cell wall degradation (Mishra et al., 2008a; Taylor et al., 1991; Tucker et al., 1991; Weaver et al., 1998) or by stimulation of ethylene production (Gepstein and Thimann, 1981). Our results suggest that senescence-promoting effects of exogenous ABA are age-dependent. To minimize visual quality loss, ABA should be applied immediately before the maturity stage, despite the relatively limited growth holding effect compared with earlier application timings.

2.5.4.4 Practical Implications

Single spray application of 3.8 mM ABA immediately before the maturity stage appears to be an effective growth holding strategy with minimal negative side effects for bell pepper transplants. Importantly, this conclusion is supported by data collected under commercial nursery conditions. Furthermore, minimal location- and cultivar-specific effects of this strategy suggest that it may be easily implemented for commercial use without extensive optimization of application methods.

In addition to prolonged transplant marketability, other beneficial consequences can be expected for this strategy. For example, suppression of excessive shoot growth can reduce maintenance costs during the extended growth period (Sharma et al., 2006b). It can also minimize damage during handling, especially when transplants are pulled from trays and packed in boxes at high density for shipment (Cantliffe, 1993). Furthermore, preferential biomass partitioning in roots may aid in improving stand establishment and subsequent field growth (Agehara and Leskovar, 2012; Garner and Björkman, 1999; Sharma et al., 2006a).

CHAPTER III

MECHANISMS OF ABSCISIC ACID-REGULATED GROWTH MODULATION

3.1 Study 6: Abscisic Acid Inhibits Leaf Expansion by Limiting Cell Expansion but not Cell Division in Arabidopsis

Abscisic acid accumulation during water stress inhibits leaf expansion to minimize increases in transpirational area. When this acclimation is induced by exogenous ABA, it has been shown previously that it is followed by rapid leaf expansion, with leaf area eventually recovering to the control level. Therefore, it was hypothesized that ABA inhibits cell expansion but not cell division, and the maintenance of cell division enables such recovery of leaf expansion after ABA degradation. To test this hypothesis, Arabidopsis plants were treated with 0 or 1 mM ABA at the rosette stage with 7–8 leaves. During 6 days following the treatment, ABA inhibited expansion of the 5th and 7th leaves by 10% and 53%, respectively, whereas it had no effect on older (1st and 3rd) leaves. Regardless of leaf age, epidermal cell number per leaf was unaffected by ABA, suggesting that ABA inhibits leaf expansion solely by limiting cell expansion. In addition, ABA affected neither number of stomata per leaf nor length of stomata, both of which regulate the rate of gas exchange and transpiration. These results suggest that ABA-induced inhibition of leaf expansion is a mechanism to conserve water without limiting plant growth capacity, as leaves maintain both cell division and stomatal formation.

3.1.1 Introduction

Leaf expansion plays a major role in plant performance. It increases light capture and CO_2 uptake required for photosynthesis, as well as transpiration that facilitates cooling and

uptake of water and nutrients (Taiz and Zeiger, 2010). The cellular basis for leaf expansion is a complex sequence of cell division and expansion (Gonzalez et al., 2012). In eudicots, leaves emerge as groups of cells that constitue leaf primordia at the periphery of the shoot apical meristem. Leaf primordia grow primarily by cell proliferation, which generates relatively small cells that remain at constant size. Cell proliferation is progressively replaced by meristemoid division as a leaf primordium develops into a leaf. Meristemoid cells are dispersed in a leaf epidermis and can form stomatal guard cells, vasucular cells, or pavement cells (Fisher and Turner, 2007; Peterson et al., 2010). At this stage, cell expansion occurs simultaneously. Meristemoid division ceases as a leaf matures, and further leaf growth is mainly achieved by cell expansion.

Because cell expansion is a turgor-driven process, leaf expansion is extremely sensitive to dehydration (Taiz and Zeiger, 2010). However, leaf growth inhibition can also occur in the absence of leaf turgor reductions during drought (Gowing et al., 1990; Passioura, 1988; Puliga et al., 1996), indicating regulatory processes that control the leaf expansion rate in response to soil drying signals rather than insufficient water. Abscisic acid is one of the chemical signals proposed to be involved in this adaptive response. Accumulation of ABA occurs in leaves under water stress (Zeevaart and Boyer, 1984; Zeevaart and Creelman, 1988). Zhang and Davies (1990a; 1990b) reported that increasing ABA concentration inhibited leaf expansion both *in vivo* and *in vitro*. Several studies found the negative association between ABA concentration and leaf expansion (Alves and Setter, 2000; He and Cramer, 1996; Van Volkenburgh and Davies, 1983).

Cellular responses to ABA may involve upregulation of potassium conductance and downregulation of proton efflux, which in turn inhibit cell expansion by membrane depolarization (Van Volkenburgh, 1999). In addition, ABA is required in the mechanism by which increased xylem sap pH inhibits cell expansion under water stress (Bacon et al., 1998). In

non-leaf tissues, increased levels of ABA also inhibits cell division (Barlow and Pilet, 1984; Myers et al., 1990). However, how ABA controls the relative contributions of cell division and expansion to leaf expansion is unknown.

In some previous observations of Study 3 and Study 4, inhibition of leaf expansion by exogenous ABA was followed by rapid leaf growth, with leaf area eventually recovering to the untreated control level. Therefore, it was hypothesized that ABA inhibits cell expansion but not cell division, and the maintenance of cell division enables such recovery of leaf expansion after ABA degradation.

3.1.2 Materials and Methods

3.1.2.1 Plant Material and Growth Conditions

The Col-0 ecotype of Arabidopsis was used in this study. Wild-type Col-0 seeds (CS60000) were obtained from the Arabidopsis Biological Resource Center. Seeds were stratified for 3 d at 4 °C and then sown in six-cell inserts filled with about 100 mL of Sunshine LC-1 soilless medium per cell. Each cell was fertilized with 5 mL of one-quarter strength Hoagland solution at 7, 14, and 17 d after sowing (DAS). Plants were grown at 25 °C under 18-h photoperiods beginning at 0800 HR with 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*.

3.1.2.2 Treatments

Stock ABA solution was prepared by dissolving (±)-cis, trans-ABA (Sigma-Aldrich, St. Louis, MO) in 100% ethanol to a concentration of 20 mM. The stock solution was diluted with de-ionized water to 1 mM and supplemented with Silwet L77 surfactant at 0.02% (v/v). The control solution was de-ionized water with the same ethanol and Silwet L77 concentrations as the ABA treatment solution but with no ABA.

At 19 DAS, when plants were at the rosette stage with 7–8 true leaves, plants were treated with 0 or 1 mM ABA solution at 100 μL/plant. A 200 μL pipette was used to apply the ABA solutions evenly over all leaves between 1100 and 1200 HR.

3.1.2.3 Leaf Replica Preparation

Four rosette leaves of different ages, from the oldest 1st, 3rd, 5th, and 7th, were sampled at 0, 1, 3, and 6 DAT. To create a replica of leaf epidermis, adaxial leaf surface was painted with clear fingernail polish. After a few minutes, the dried nail polish patch was peeled off using a piece of clear cellophane tape and attached on a microscope slide (Ted Pella, Redding, CA).

3.1.2.4 Leaf Area

Immediately after leaf replica preparation, leaves were scanned using a flat-bed scanner at 600 dpi. Leaf area was calculated using ImageJ image processing software (http://rsb.info.nih.gov/ij/).

3.1.2.5 Size and Number of Epidermal Cells and Stomata

Bright-field microscopy was performed using a Zeiss Axiopot microscope (Zeiss, Thornwood, NY) with a 10×/0.3 numerical aperture (NA) objective lens (Plan Neofluar; Zeiss) to obtain five magnified images from each leaf replica. Number of epidermal cells and stomata were counted in a 400 × 400 μm zone selected from each image, avoiding major veins or trichomes. Total number of epidermal cells and stomata per leaf were estimated from cell or stomatal density and leaf area. Stomatal guard cell length was measured on 25 stomata per leaf using ImageJ software.

3.1.2.6 Statistical Design and Analysis

Treatments were factorial combinations of two ABA concentrations (0 and 1 mM) and four leaf ages (1st, 3rd, 5th, and 7th). There were five replicates (plants) for each treatment arranged in a split-plot design, with ABA concentration as the main plot and leaf age as the subplot. All data analyses were run in SAS, and *P* values less than 0.05 were considered statistically significant. Treatment and interaction effects were tested using the restricted maximum likelihood method with the DDFM=KR option in the MIXED procedure. Multiple comparisons of least squares means were performed by the Tukey–Kramer test in the MIXED procedure. When heteroscedasticity was indicated by a likelihood ratio test, the MIXED procedure was run with the GROUP option in the REPEATED statement.

3.1.3 Results and Discussion

3.1.3.1 Abscisic Acid-induced Inhibition of Leaf Expansion is Leaf Age-dependent

Leaf expansion occurred more rapidly in younger leaves (Figs. 3.1A and 3.2). During the 6-d measurement period, increases in leaf area of the untreated plants were 8% in the 1st (oldest) leaf (0.33 vs. 0.35 cm^2), 45% in the 3rd leaf (1.02 vs. 0.70 cm^2), 126% in the 5th leaf (0.93 vs. 2.10 cm^2), and 524% in the 7th leaf (0.52 vs. 3.27 cm^2) (Fig. 3.1A). Similarly, ABA-induced inhibition of leaf expansion occurred more severely in younger leaves. At 6 DAT, reductions in leaf area by exogenous ABA were 0% in the 1st leaf (0.35 vs. 0.35 cm^2), 5% in the 3rd leaf (1.02 vs. 0.96 cm^2), 10% in the 5th leaf (2.10 vs. 1.88 cm^2), and 53% in the 7th leaf (3.27 vs. 1.54 cm^2). These reductions were statistically significant only for the 1st leaf. The lack of statistical significance for older leaves were due likely to their small amount of potential growth during the measurement period. Stomata are known to be preferential sites for foliar absorption (Mansfield et al., 1983). At the time of ABA treatment, stomata were denser in

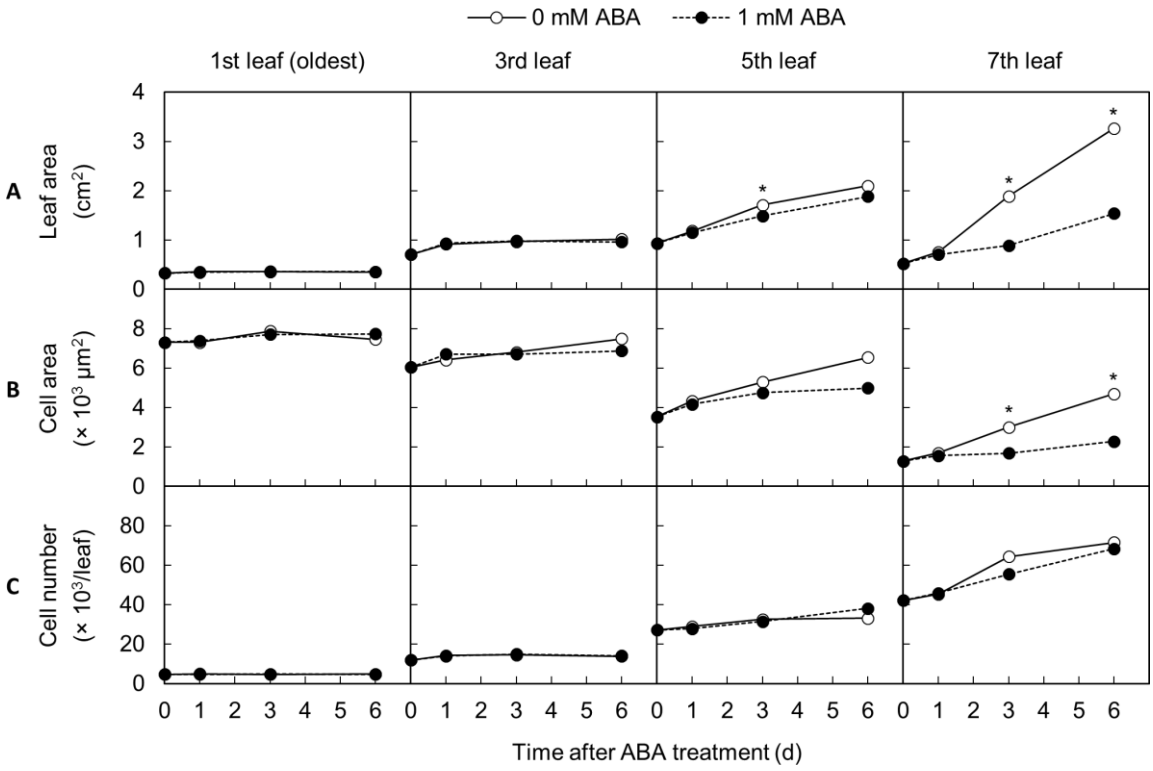

Fig. 3.1. Leaf expansion as a function of cell division and expansion in four Arabidopsis rosette leaves of different ages as affected by exogenous abscisic acid (ABA) (Study 6): (**A**) leaf area, (**B**) average area of pavement cells in adaxial epidermis, and (**C**) number of pavement cells in adaxial epidermis. Plants were treated with ABA at the rosette stage with 7-8 leaves. Asterisks indicate significant differences (Tukey–Kramer test, $P < 0.05$).

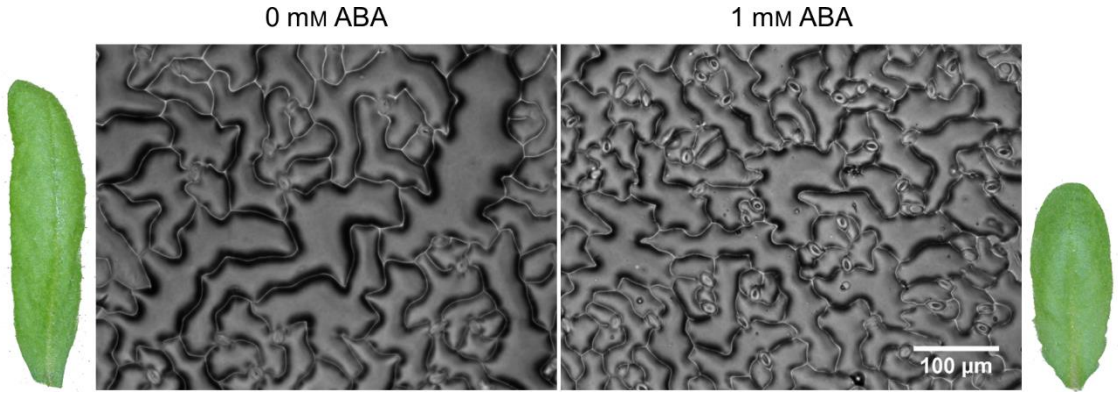

Fig. 3.2. Photographs of Arabidopsis rosette leaves and bright-field micrographs of adaxial epidermis 6 d after abscisic acid (ABA) treatment (Study 6). Plants were treated with ABA at the rosette stage with 7-8 leaves. Images were obtained from the 7th rosette leaf, which had the most rapid growth during the experiment.

younger leaves by up to 8 times compared with the oldest leaf (46 vs. 373 per mm^2) (Figs. 3.3A), suggesting that younger leaves have higher absorption capacity. By contrast, mature leaves typically develop thicker leaf cuticles, which prevent penetration of water and solutes (Hull, 1970).

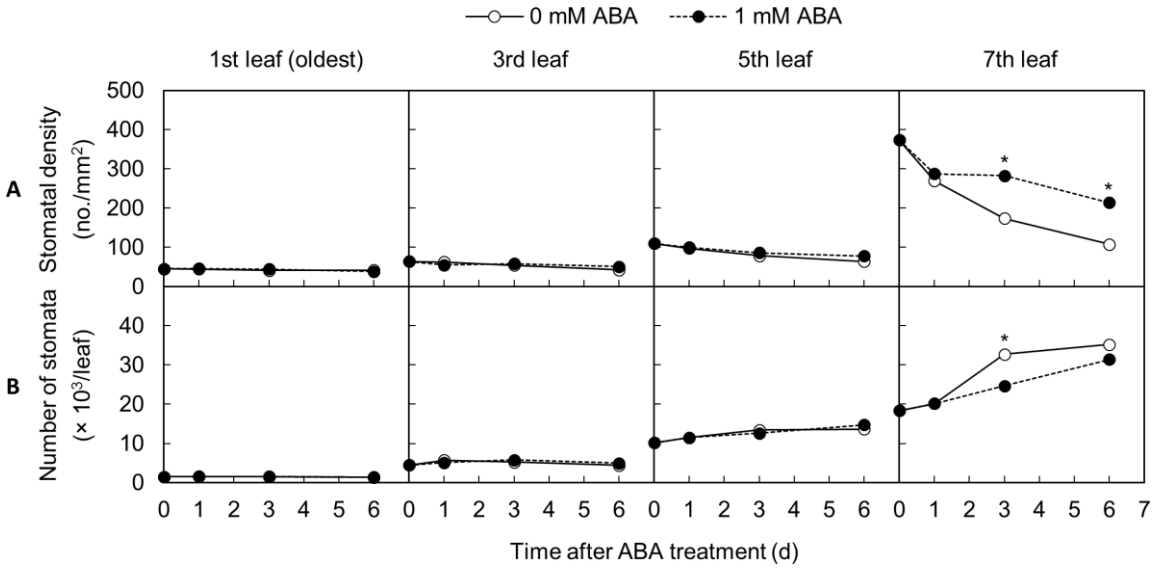

Fig. 3.3. Stomatal development in four Arabidopsis rosette leaves of different ages as affected by exogenous abscisic acid (ABA) (Study 6): (**A**) stomatal density in adaxial epidermis and (**B**) number of stomata in adaxial epidermis. Plants were treated with ABA at the rosette stage with 7-8 leaves. Asterisks indicate significant differences (Tukey–Kramer test, $P < 0.05$).

3.1.3.2 Abscisic Acid Inhibits Leaf Expansion by Limiting Cell Expansion but Not Cell Division

Although increased levels of ABA inhibit cell division and expansion in several tissue types (Bacon et al., 1998; Barlow and Pilet, 1984; Myers et al., 1990), how ABA controls their relative contributions to leaf expansion is unknown. In this study, exogenous ABA reduced epidermal cell area of the 7th leaf by 51% at 6 DAT (4690 vs. 2279 μm^2) (Figs. 3.1B and 3.2), whereas it had no effect on cell division regardless of leaf age (Fig. 3.1C). Furthermore, the magnitude of ABA-induced inhibition was very similar in leaf expansion and cell expansion

(Fig. 3.1A and B). These results suggest that ABA inhibits leaf expansion solely by limiting cell expansion.

During leaf development, cell division consists of two partially overlapping phases (Gonzalez et al., 2012). First, cell proliferation occurs in a leaf primordium to rapidly generate relatively small cells that remain at constant size. Cell proliferation is progressively replaced by meristemoid division as a leaf primordium develops into a leaf. At this stage, cell expansion occurs simultaneously. Meristemoid division ceases before cell expansion as a leaf matures. Considering the initial leaf sizes, most measurements in this study were likely performed in the meristemoid division phase. Because meristemoid cells undergo three sequential asymmetric divisions, they can generate nearly half of the pavement cells in a leaf (Bergmann and Sack, 2007; Geisler et al., 2000). In fact, cell numbers in the 7th leaf of the untreated plants increased by 70% (42126 vs. 71604 per cell) during the measurement period. The lack of inhibitory effect of ABA on meristemoid division is important to maintain leaf growth capacity and facilitate rapid leaf expansion upon ABA degradation.

3.1.3.3 Stomatal Formation Is Minimally Affected by Abscisic Acid

The effect of ABA on stomatal formation was minimal; exogenous ABA only transiently inhibited stomatal formation (Fig. 3.3B) and had no significant effect on stomatal guard cell length (data not shown). Because exogenous ABA did not increase number of stomata, increased stomatal density in the 7th leaf by exogenous ABA (Fig. 3.3A) was due simply to the reduction in leaf area (Fig. 3.1A). Stomatal guard cells are formed from meristemoid cells during the cell expansion phase (Peterson et al., 2010). These results suggest that ABA only transiently arrest differentiation of meristemoid cells to stomata.

Both number and size of stomata are important variables that regulate the rate of CO_2 entry and thus photosynthesis (Lawlor, 2002). Therefore, the minimal effect of ABA on stomatal formation is important to maintain high photosynthetic capacity. Furthermore, the results in this study collectively suggest that ABA-induced inhibition of leaf expansion is a mechanism to conserve water without limiting plant growth capacity, as leaves maintain both cell division and stomatal formation.

3.2 Study 7: Abscisic Acid Limits Nitrogen Distribution to Matured Leaves and Induces Leaf Age-dependent Chlorosis in Arabidopsis

Leaf chlorosis induced by high-dose ABA application is a limiting factor for its commercial use. This study examined effects of ABA on ethylene synthesis and nutrient uptake and distribution as contributing factors for ABA-induced chlorosis. Arabidopsis plants were treated with 0, 0.1, or 1 mM ABA at the rosette stage with 7–8 leaves. Four leaves of different maturity stages were used for all measurements: 1st (oldest), 3rd, 5th, and 7th leaves. Chlorosis occurred mainly in the oldest leaf treated with 1 mM ABA; leaf chlorophyll index (SPAD reading) decreased by 44% within 24 hours of treatment and by up to 78% thereafter. As opposed to the general assumption that ABA-induced chlorosis is mediated by senescing-effects of ethylene, 1 mM ABA significantly suppressed ethylene synthesis shortly after treatment. Uptake and distribution of N was traced using ^{15}N-labeled KNO_3 added to growth medium immediately after ABA treatment. There was a strong positive correlation between leaf dry matter production and $\delta^{15}N$ ($r^2 = 0.703$), suggesting that in most cases $\delta^{15}N$ increased proportionally to new growth. However, $\delta^{15}N$ decreased with increasing ABA concentration in the oldest leaf, which had no new growth and thus required N only for maintenance during the experiment. These results suggest that ABA

limits distribution of N into non-growing matured leaves, thereby inducing leaf-age dependent chlorosis.

3.2.1 Introduction

Leaf chlorosis is reported as a negative side effect of ABA application in many vegetable and ornamental crops, limiting its potential for commercial use (Agehara and Leskovar, 2012; Kim and van Iersel, 2011; Waterland et al., 2010c). Therefore, understanding the mechanisms by which ABA stimulate leaf chlorosis is important to develop a specific application strategy with minimum undesirable effects.

Ethylene is also known to play a role in leaf senescence, and ABA can promote leaf senescence through stimulation of ethylene production (Gepstein and Thimann, 1981). However, effects of ABA on leaf senescence are not fully mediated by ethylene (Zacarias and Reid, 1990). This ethylene-independent effect of ABA was demonstrated by Zacarias and Reid (1990), who compared the leaf senescing effects of ABA and ethylene. When leaf discs of Arabidopsis wild type and the ethylene insensitive mutant were treated with ethylene, chlorophyll loss was accelerated on the wild-type leaf discs, but no yellowing was observed on the leaf discs of the ethylene insensitive mutant. By contrast, ABA treatment stimulated chlorosis in both wild-type and mutant leaf discs.

The ABA-induced chlorosis can be attributed to the senescing effects of ABA, resulting from the expression of hydrolytic enzymes involved in chlorophyll breakdown (Weaver et al., 1998). Expression of several senescence associated genes (Weaver et al., 1998) and H_2O_2 accumulation (Hung and Kao, 2004) are also ABA-inducible and can promote leaf senescence. Although these regulatory mechanisms are well identified in recent genetic studies (Buchanan-Wollaston et al., 2005; Lee et al., 2011; Lim et al., 2007), most data were obtained with excised

leaf tissues. Nutrient deficiency is another factor that can promote leaf chlorosis (Lim et al., 2007). In intact plants, ABA may also stimulate leaf chlorosis by inducing stomatal closure and limiting transpiration-driven mass flow of N. The objective of this study was to examine effects of ABA on ethylene synthesis and nutrient uptake as contributing factors for ABA-induced chlorosis.

3.2.2 Materials and Methods

3.2.2.1 Plant Material and Growth Conditions

Arabidopsis Col-0 was grown as described for Study 6, except that each plant was fertilized with 10 mL of fertilizer solution (20N–20P–20K) at 200 mg of each nutrient per liter at 7 and 14 DAS.

3.2.2.2 Abscisic Acid Treatment and ^{15}N Labelling

At 19 DAS, when plants were at the rosette stage with 7–8 true leaves, plants were treated with 0, 0.1, or 1 mM ABA solution at 100 µL/plant. The test solutions were prepared and applied as described for Study 6.

To examine the effects of ABA on N uptake and translocation, each pot was fertilized with 10 mL of 5 atom% ^{15}N-KNO_3 (Sigma-Aldrich) solution at 200 mg $N \cdot L^{-1}$ immediately after ABA treatment. The medium surface was covered with a polystyrene disk prior to ^{15}N application to avoid the contact of leaves with ^{15}N.

3.2.2.3 Ethylene Measurement

Four rosette leaves of different ages, including 1st (oldest), 3rd, 5th, and 7th, were sampled between 1100 and 1200 HR at 0, 1, 2, 3, and 5 DAT. Each leaf lamina was cut at the

petiole attachment point and placed in a 3-mL plastic vessel connected to a 1-mL syringe through a 3-way valve. Five sets of 3-mL vessel and 1-mL syringe without leaves were also prepared to account for background ethylene. Samples were kept under the plant growth condition for 20 min. Thereafter, 1 mL of gas was transferred from the vessel to the syringe and analyzed on a 10SPlus gas chromatograph (Photovac, Markham, Ontario, Canada) with a 3.2 mm × 124 cm Carbopak BHT column and a 43 cm pre-column. The ethylene synthesis rate was calculated on a fresh weight (FW) basis.

3.2.2.4 Leaf Chlorophyll Index

Immediately after ethylene measurement, leaf chlorophyll index was measured using a chlorophyll meter (SPAD-502; Konica Minolta Sensing). Two readings were taken per leaf, avoiding major leaf veins.

3.2.2.5 Isotopic Analysis

After sampling four leaves, laminae of remaining rosette leaves were separated from stems and petioles. Consequently, there were six plant tissue samples for each plant: laminae of 1st, 3rd, 5th, and 7th leaves, laminae of all other leaves, and stems with petioles. These samples were separately dried at 65 °C for 48 h to determine dry weight and stored in capped glass vials until isotopic analysis was performed. Dried tissue samples from 3 DAT were cut in the vials using scissors to fine pieces and analyzed for total N concentration and $\delta^{15}N$ at the University of Arkansas Stable Isotope Laboratory (Fayetteville, AR).

3.2.2.6 Statistical Design and Analysis

Treatments were factorial combinations of three ABA concentrations (0, 0.1, and 1 mM) and four leaf ages (1st, 3rd, 5th, and 7th). There were four replicates (plants) for each treatment arranged in a split-plot design, with ABA concentration as the main plot and leaf age as the subplot. All data analyses were run in SAS as described for Study 6.

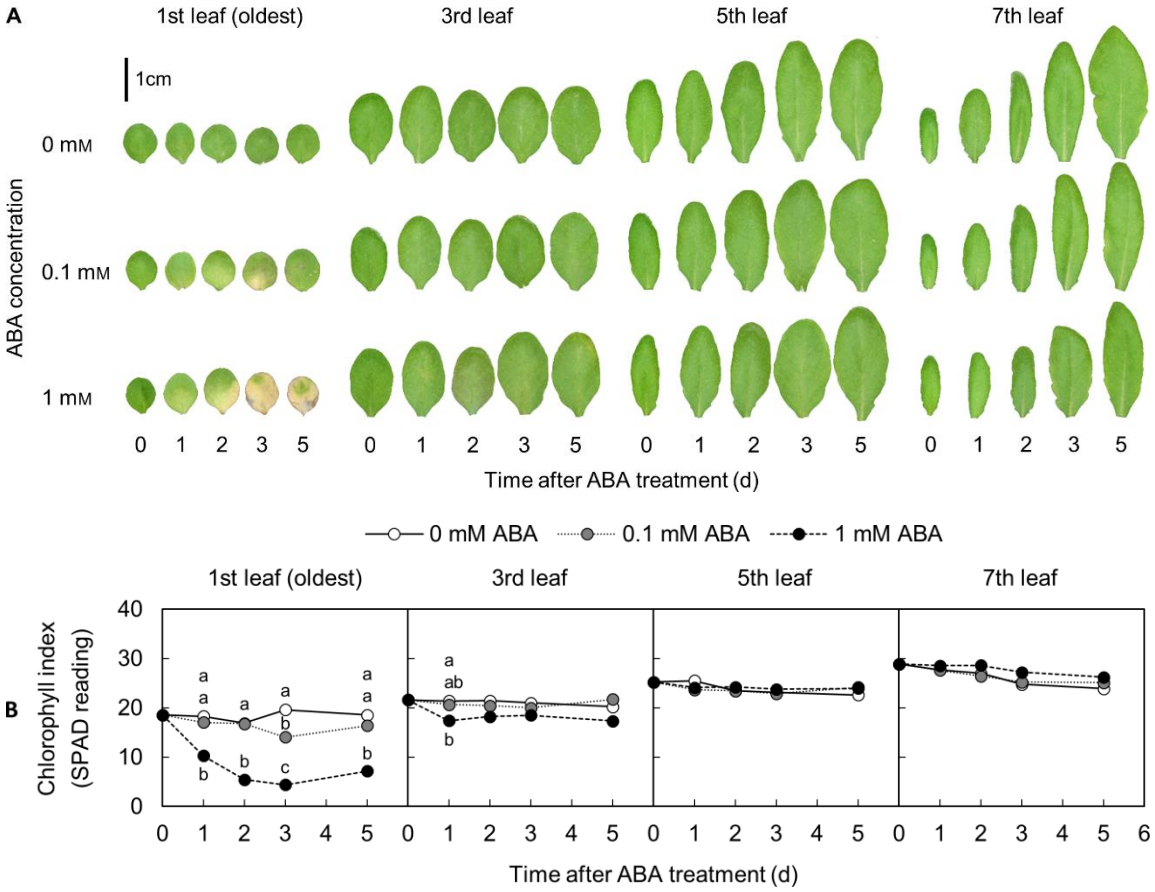

Fig. 3.4. Leaf chlorosis in four Arabidopsis rosette leaves of different ages as affected by exogenous abscisic acid (ABA) (Study 7): (**A**) chlorosis and (**B**) leaf chlorophyll index. Plants were treated with ABA at the rosette stage with 7-8 leaves. For each leaf age and measurement time, means (n = 4) of chlorophyll index (**B**) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

3.2.3 Results and Discussion

3.2.3.1 Abscisic Acid-induced Chlorosis Is Leaf Age-dependent

Leaf chlorosis was visible and also indicated by reductions in chlorophyll index in the ABA-treated leaves (Fig. 3.4A and B). The magnitude of chlorosis was pronounced with increasing ABA concentration and leaf maturity. In the 1st leaf, 1 mM ABA treatment reduced chlorophyll index by 44% at 1 DAT (18.3 vs. 10.3) and by up to 78% thereafter (19.6 vs. 4.4). In some cases, severe chlorosis on this leaf progressed to necrosis. By contrast, chlorosis induced by 0.1 mM ABA on the 1st leaf and by 1 mM ABA on the 3rd leaf was moderate and reversible. Neither 0.1 nor 1 mM ABA treatment induced chlorosis on the 5th and 7th leaves. These results suggest that ABA induces leaf chlorosis more severely in more matured leaves. Similar leaf age-dependent chlorosis in response to exogenous ABA has been observed in several crops (Waterland et al., 2010b; Waterland et al., 2010c).

3.2.3.2 Abscisic Acid Induces Chlorosis Independently of Ethylene Synthesis

Because of lack of significant leaf age × ABA concentration interaction (data not shown), ethylene data were pooled by each main effect. The ethylene synthesis rate in the 1st and 7th leaves was relatively high at 0 and 1 DAT, ranging from 56 to 79 and 56 to 98 pmole·g^{-1} FW·d^{-1}, respectively, whereas in the other leaves it remained below 40 pmole·g^{-1} FW·d^{-1} throughout the experiment (Fig. 3.5). In general, ethylene synthesis started to decline at 1 DAT and showed only minor differences by leaf age thereafter. These variations in ethylene synthesis by leaf age appeared to have no correlation with ABA-induced chlorosis (Fig. 3.4).

Averaging across leaf ages, ethylene synthesis in the untreated leaves started to decline at 1 DAT and remained low after 3 DAT (Fig. 3.5). The declining rate was accelerated by ABA

treatment. As a result, ethylene synthesis was suppressed by up to 55% in the 1 mM ABA-treated leaves at 2 DAT (39 vs. 18 pmole·g^{-1} FW·d^{-1}).

One mechanism generally proposed for ABA-induced chlorosis is senescence by stimulated synthesis of ethylene (Gepstein and Thimann, 1981). The results in this study were contrary to this general assumption, and suggest that other mechanisms are involved in the observed ABA-induced chlorosis.

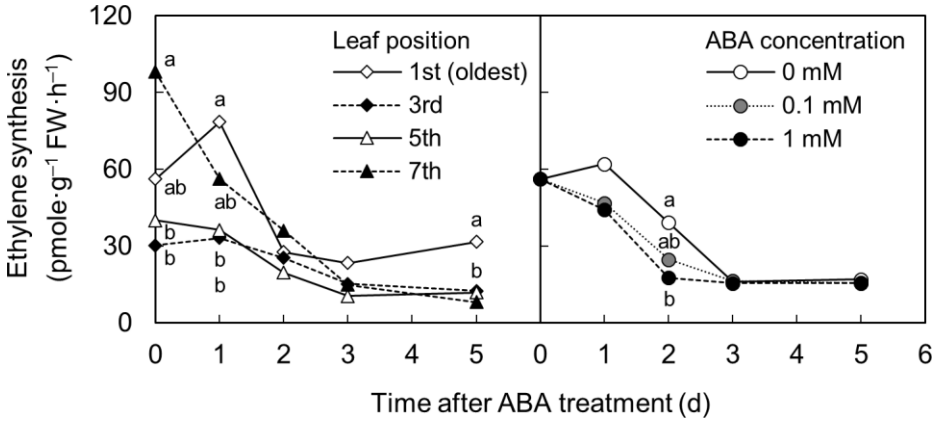

Fig. 3.5. Ethylene synthesis in four Arabidopsis rosette leaves of different ages as affected by exogenous abscisic acid (ABA) (Study 7). Plants were treated with ABA at the rosette stage with 7-8 leaves. For each leaf age and measurement time, means (n = 4) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). FW = fresh weight.

3.2.3.3 Abscisic Acid Limits Nitrogen Distribution to Matured Leaves and Induces Leaf Age-dependent Chlorosis

In general, dry weight, N concentration and content, and $\delta^{15}N$ measured in leaves at 3 DAT decreased with increasing ABA concentration and leaf age (Table 3.1). Because transpiration rate also decreased within increasing ABA concentration (Fig. 3.6), the reductions in dry matter production and N uptake by ABA are due likely to stomatal closure that limits

Table 3.1. Dry weight, N concentration and content, and $\delta^{15}N$ in four Arabidopsis rosette leaves of different maturity stages 3 d after abscisic acid (ABA) treatment (Study 7).

	ABA[z]	Dry wt[y]	N		$\delta^{15}N$
Leaf position	(mM)	(mg)	(%)	(μg)	(‰)
1st (oldest)	0.0	0.73 aC[x]	5.46 aB	41 aC	711 aC
	0.1	0.77 aB	4.84 aB	38 aC	599 aC
	1.0	0.59 aB	3.83 bC	22 bC	372 bB
3rd	0.0	2.25 aB	5.66 aAB	127 aB	919 aBC
	0.1	2.39 aA	5.67 aAB	135 aB	917 aB
	1.0	2.10 aA	5.25 aB	110 aB	515 bAB
5th	0.0	3.37 aA	6.12 aAB	206 aA	1134 aB
	0.1	3.21 aA	6.19 aAB	198 aA	1042 aAB
	1.0	2.89 aA	5.91 aAB	171 aA	663 bAB
7th	0.0	3.81 aA	6.68 aA	254 aA	1497 aA
	0.1	3.07 abA	6.38 aA	196 bA	1238 aA
	1.0	2.74 bA	6.09 aA	167 bA	794 bA

[z]Plants were treated with ABA at the rosette stage with 7-8 leaves.
[y]Dry weight of the 1st, 3rd, 5th, and 7th leaves measured immediately before ABA treatment was 0.73, 1.81, 2.14, and 1.36 mg, respectively.
[x]Means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

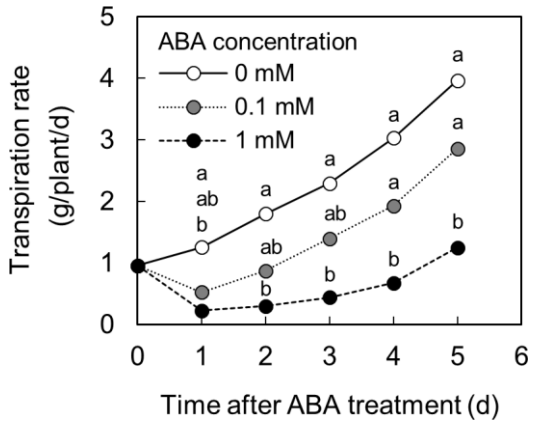

Fig. 3.6. Transpiration rate of Arabidopsis plants as affected by exogenous abscisic acid (ABA) (Study 7). Plants were treated with ABA at the rosette stage with 7-8 leaves. At each measurement time, means (n = 4) with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

photosynthesis (Amthor, 2007; Davies and Jones, 1991) and transpiration-driven mass flow of N (Havlin et al., 1999).

There was a strong positive correlation between leaf dry matter production (leaf dry matter increase from 0 to 3 DAT) and $\delta^{15}N$ ($r^2 = 0.703$) (Fig. 3.7), suggesting that, in most cases, $\delta^{15}N$ increased proportionally to new growth. However, $\delta^{15}N$ decreased with increasing ABA concentration even in the oldest leaf, which had no new growth and thus required N mainly for the maintenance of chlorophyll and proteins during the experiment. These results suggest that ABA limits distribution of N into non-growing matured leaves, thereby inducing leaf-age dependent chlorosis.

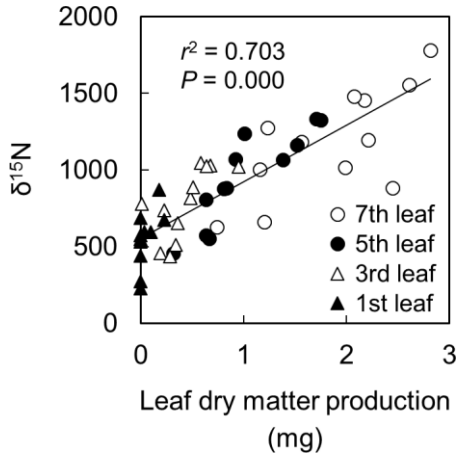

Fig. 3.7. Linear association between leaf dry matter production and $\delta^{15}N$ in Arabidopsis leaves (Study 7). Data include 12 treatments in factorial combinations of four leaf ages (1st = oldest) and three abscisic acid (ABA) concentrations (0, 0.1, and 1 mM) with four replications. Plants were treated with ABA at the rosette stage with 7-8 leaves. Leaves were sampled for measurements 3 d after ABA treatment (DAT). Leaf dry matter production = leaf dry weight at 3 DAT – leaf dry weight at 0 DAT.

3.3 Study 8: Promotive Effect of Abscisic Acid on Primary Root Elongation Is Not Associated with Protection of Root Tip Structures from Dehydration-induced Damage in Arabidopsis

Accumulation of ABA in root tips is required for the maintenance of primary root elongation during water stress. The objective of this study is to examine if the promotive effect of ABA on root growth is associated with protection of root tip structures from dehydration-induced damage. Arabidopsis wild-type Col-0 and two ABA-deficient mutant lines, *aba2-1* and *nced3-2*, were grown for 5 d in basal agar medium, and then grown for additional 5 d in agar media with different levels of water potential (–0.1, –1.0, and –1.7 MPa) and ABA (0, 1, and 10 μM). All three lines showed similar sensitivity to water stress. However, the promotive effect of 1 μM ABA treatment on root elongation was pronounced by deficiency of endogenous ABA in the two mutants. First, at –0.1 MPa, the increase in root elongation rate by 1 μM ABA was 14% in Col-0 and 31% in *aba2-1*. Second, at –1.0 MPa, the increase in root elongation rate by 1 μM ABA was 24% in Col-0 and 96% in *nced3-2*. These increases were statistically significant only for the mutants. In addition, increasing ABA concentration from 1 to 10 μM significantly inhibited root elongation of Col-0, whereas it only lessened the promotive effect of ABA in the mutants. These results suggest that increased levels of ABA can promote primary root elongation, particularly when plants are under water stress. However, root tip morphology visualized by SEM revealed that the promotive effect of ABA was not due to protection of root tips from dehydration-induced damage.

3.3.1 Introduction

Roots continue to grow under water deficit conditions that severely inhibit shoot growth (Sharp and Davies, 1979; Westgate and Boyer, 1985). This differential growth response of roots and shoots can improve the balance between water uptake and transpiration, thereby helping plants cope with water stress. The maintenance of root elongation under water stress is particularly important for transplanted vegetable seedlings, which must quickly overcome transplant shock to re-establish normal growth. Therefore, understanding the mechanisms of root growth acclimation to water stress is important for improving plant performance under water-limited conditions.

The role of ABA in primary root elongation under water deficit has been studied extensively in maize. Saab et al. (1990) proposed that ABA accumulation in root tips is required for the maintenance of primary root elongation under water deficit conditions. Their approach was to induce ABA deficiency by using fluridone, which limits ABA precursors by inhibiting carotenoid biosynthesis, or by using a mutant, in which carotenoid biosynthesis is deficient. Inhibition of ABA accumulation by either method resulted in severe reductions in root elongation at low water potential. This finding was confirmed in a subsequent study that showed a full recovery of root elongation when ABA in the elongation zone was restored to normal levels with exogenous ABA (Sharp, 1994). Furthermore, Sharp (2002) suggested that an important role of ABA in the maintenance of root elongation is to limit ethylene production.

Cell wall acts as an important site of defense against desiccation (Hoson, 1998). Cell wall compositions in roots change in response to water stress. Leucci et al. (2008) compared cell wall polysaccharides in apical root zones of two wheat cultivars varying in drought sensitivity, and found the accumulation of pectin side chains in response to water stress only in a drought-tolerant cultivar. Pectin side chains such as arabinans and galactans can improve hydration status

of cell wall matrix because of their high water binding capability and ability to form gells (Willats et al., 2001). These hydration forces play an important role in protecting symplast during water deficit (Leucci et al., 2008).

The fact that accumulation of ABA is required for the maintenance of root elongation under water deficit may indicate that ABA plays a role in protecting root tips by enhancing the strength of cell structures. To test this hypothesis, SEM was used in this study to visualize Arabidopsis root tips subjected to water stress and exogenous ABA.

3.3.2 Materials and Methods

3.3.2.1 Plant Material

The Col-0 ecotype of Arabidopsis was used in this study. Wild-type Col-0 and two ABA deficient lines, *aba2-1* and *nced3-2*, were used. The *nced3-2* mutant has been described by Urano et al. (2009).

3.3.2.2 Abscisic Acid and Water Stress Treatments

Treatments of ABA and water stress were performed using vertically-positioned agar plates as described by Verslues and Bray (2006), with modifications. Seeds were stratified for 3 d at 4 °C and then plated in 10-cm plates containing 20 mL of basal medium (half-strength Murashige and Skoog salts, 6 mM morpholineethanesulfonic acid buffer, and 1% agar with pH 5.7). Prior to seed plating, each plate was overlain with a cellophane membrane sheet (Gel Company, San Francisco, CA) to facilitate transfer of seedlings to a new plate. Plates were placed vertically and kept at 25 °C under 18-h photoperiods beginning at 0800 HR with 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* . After 5 d, seedlings were transferred using the cellophane sheets to new plates for ABA and water stress treatments.

For ABA treatments, 20 mM ABA dissolved in 100% ethanol was added to autoclaved basal medium before solidification to adjust medium ABA concentrations to 1 and 10 μM. Additional 100% ethanol was added to 0 μM ABA basal medium and 1 μM ABA medium to equilibrate ethanol concentration in all media.

To prepare low water potential media, polyethylene glycol (PEG) (PEG 8000; VWR International) was infused into the ABA media by overlaying 30 mL of PEG solution over 20 mL of each medium in a 10-cm plate. After 15 h, overlaying PEG solution was removed. Water potential was adjusted by the PEG concentration used; 0, 400, 500 g of PEG per liter produced medium water potential of –0.1, –1.0, and –1.7 MPa, respectively. Water potential was measured using a vapor pressure osmometer (HR-33T; Wescor, Logan, UT).

3.3.2.3 Root Length and Lateral Root Number

Plates were photographed 5 d after transfer of seedlings to the treatment media. Primary root length and lateral root number were determined from the images using ImageJ software.

3.3.2.4 Scanning Electron Microscopy

Scanning electron microscopy was performed on roots subjected to water stress and ABA treatments for 5 d. Seedling roots were fixed with vapor acrolein (VWR International) for 6 h at 23 °C, transferred to 100% hexamethyldisilazane (VWR International) for 24 h, gradually dehydrated, and mounted on stubs with double-sided sticky carbon tape. Mounted specimens were coated with gold using a sputter coater (Cressington 108; Cressington Scientific Instruments, Watford, UK) and imaged using a JEOL6400 (JEOL, Tokyo, Japan) with a secondary electron detector at 10 kV. Working distance was 48 mm.

3.3.2.5 Statistical Design and Analysis

Treatments were factorial combinations of three water potentials (–0.1, –1.0, and –1.7 MPa) and three ABA concentrations (0, 1, and 10 μM) arranged in a completely randomized design. Each treatment combination was randomly assigned to 27 petri dishes to provide three replications. Within each petri dish, three Arabidopsis lines (Col-0, *aba2-1*, and *nced3-2*) were randomized with four plants per line. All data analyses were run in SAS as described for Study 6, except that pre-treatment root length was included as a covariate when analyzing root elongation rate.

3.3.3 Results and Discussion

3.3.3.1 Abscisic Acid Promotes Primary Root Elongation

The role of ABA in primary root elongation under water deficit has been studied extensively in maize. Saab et al. (1990) proposed that ABA accumulation in root tips is required for the maintenance of primary root elongation under water deficit conditions. Their approach was to induce ABA deficiency by using fluridone, which limits ABA precursors by inhibiting carotenoid biosynthesis, or by using a mutant, in which carotenoid biosynthesis is deficient. Inhibition of ABA accumulation by either method resulted in severe reductions in root elongation at low water potential. This finding was further confirmed in a subsequent study that showed a full recovery of root elongation when ABA in the elongation zone was restored to normal levels with exogenous ABA (Sharp, 1994).

In this study, wild-type Col-0 and two ABA-deficient mutant plants were exposed to moderate (–1.0 MPa) and severe (–1.7 MPa) water stress using the PEG-infused growth media. Despite the suggested essential role of ABA in maintaining root growth under water deficit (Saab et al., 1990; Sharp, 1994; Sharp et al., 2004), all three lines showed similar sensitivity to

water stress; at 0 μM ABA, reductions in root elongation rate with lowering water potential from –1 to –1.0 MPa were 69% to 72%, and those from –0.1 to –1.7 MPa were 84% to 88% (Fig. 3.8). However, the promotive effect of 1 μM ABA treatment on root elongation was enhanced by deficiency of endogenous ABA in the two mutants. First, at –0.1 MPa, the increase in root elongation rate by 1 μM ABA was 14% in Col-0 (5.81 vs. 6.62 mm·d^{-1}) and 31% in *aba2-1* (4.92 vs. 6.43 mm·d^{-1}). Second, at –1.0 MPa, the increase in root elongation rate by 1 μM ABA was 24% in Col-0 (2.38 vs. 2.95 mm·d^{-1}) and 96% in *nced3-2* (1.27 vs. 2.49 mm·d^{-1}). These increases were statistically significant only for the mutants. In addition, increasing ABA concentration from 1 to 10 μM significantly inhibited root elongation of Col-0, whereas it only lessened the promotive effect of ABA in *aba2-1* and *nced3-2*. These results suggest that increased levels of ABA can promote primary root elongation, particularly when plants are under water stress.

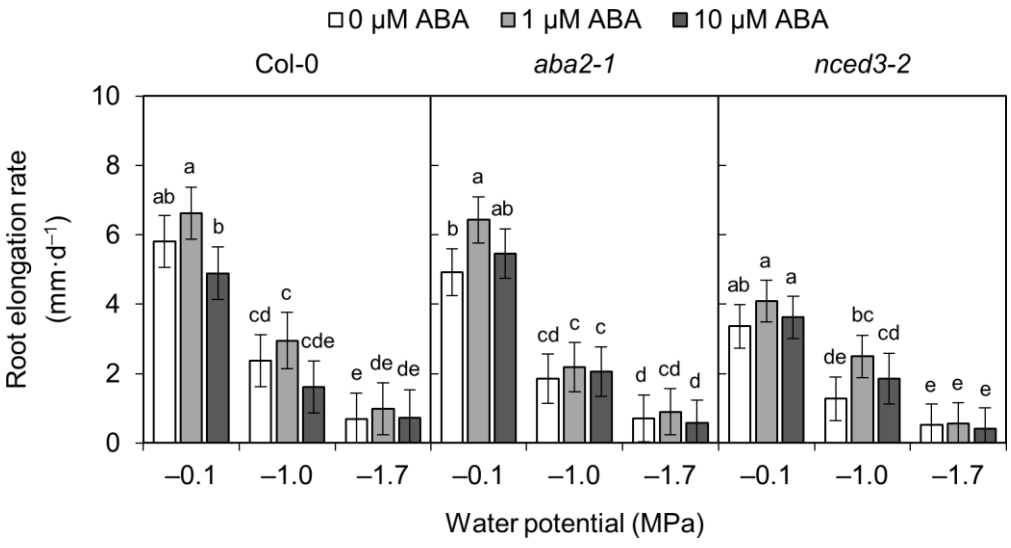

Fig. 3.8. Primary root elongation of Arabidopsis wild-type Col-0, *aba2-1*, and *nced3-2* plants grown in agar media for 5 d with different water potentials and abscisic acid (ABA) concentrations (Study 8). Data are means ± 95% confidence intervals (n = 4). For each line, bars with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

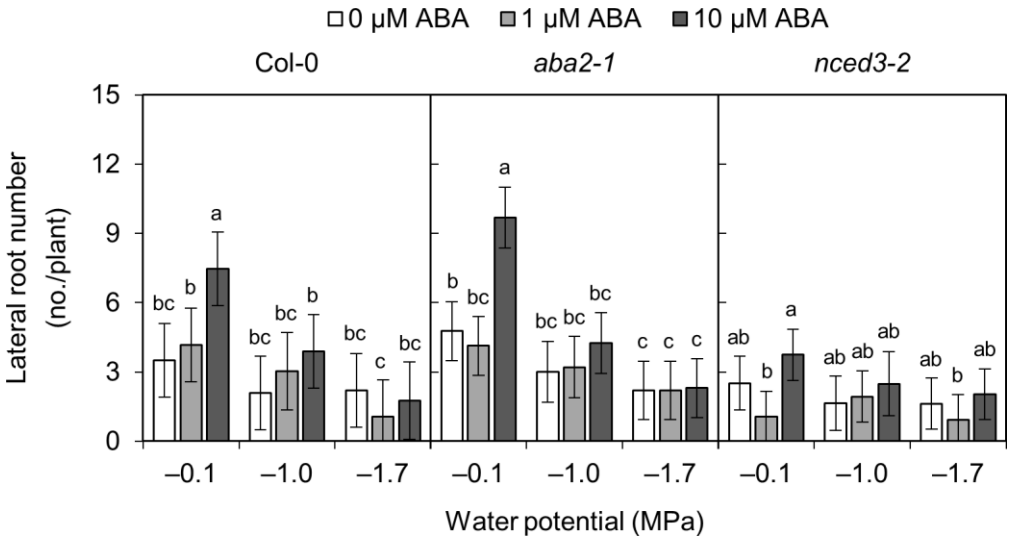

Fig. 3.9. Lateral root formation of Arabidopsis wild-type Col-0, *aba2-1*, and *nced3-2* plants grown for 5 d in agar media with different water potentials and abscisic acid (ABA) concentrations (Study 8). Data are means ± 95% confidence intervals (n = 4). For each line, bars with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

3.3.3.2 Abscisic Acid Stimulates Lateral Root Formation in the Absence of Water Stress

In Arabidopsis, prolonged water stress induces development of short lateral roots, characterized by stubby tuberized structures (Vartanian et al., 1994). These specialized lateral roots enter a dormant mode and resume growth upon rehydration. This adaptive process is severely compromised in ABA-insensitive mutants such as *abi1-1*, suggesting that ABA is involved in the signaling of lateral root development.

In this study, neither 1 nor 10 µM ABA exerted significant effect on lateral root formation at low water potentials, but 10 µM ABA increased lateral root number of Col-0 and *aba2-1* by 114% (3.49 vs. 7.46) and 103% (4.77 vs. 9.68), respectively (Fig. 3.9). Inhibition of lateral root formation is an adaptive response to water stress (Xiong et al., 2006). Therefore, water stress-regulated inhibition may have masked the promotive effect of ABA on lateral root development. Unlike primary root elongation, lateral root formation showed no inhibition in

response to 10 μM ABA. This observation, along with the lack of distinct response to ABA in the ABA deficient mutants, may indicate that primary root tips and the sites of lateral root initiation have different sensitivity to ABA.

3.3.3.3 Abscisic Acid Does Not Alleviate Dehydration-induced Damage on Root Tip Structure

Dehydration-induced structural damage on root tips was visualized by SEM (Fig. 3.10). In all three lines (images not shown for *aba2-1*), such damage was consistently characterized by thickening and deformation of root tips. There was no visual difference in the degree of damage between the –1.0 and –1.7 MPa treatments (images not shown for –1.7 MPa). Similar root tip structural damage is reported for weeping grass [*Microlaena stipoides* (Labill.) R.Br.] grown under high-strength soils.

The results in this study suggest that the promotive effect of ABA on primary root elongation under water stress is not associated with protection of root tips from dehydration-induced damage.

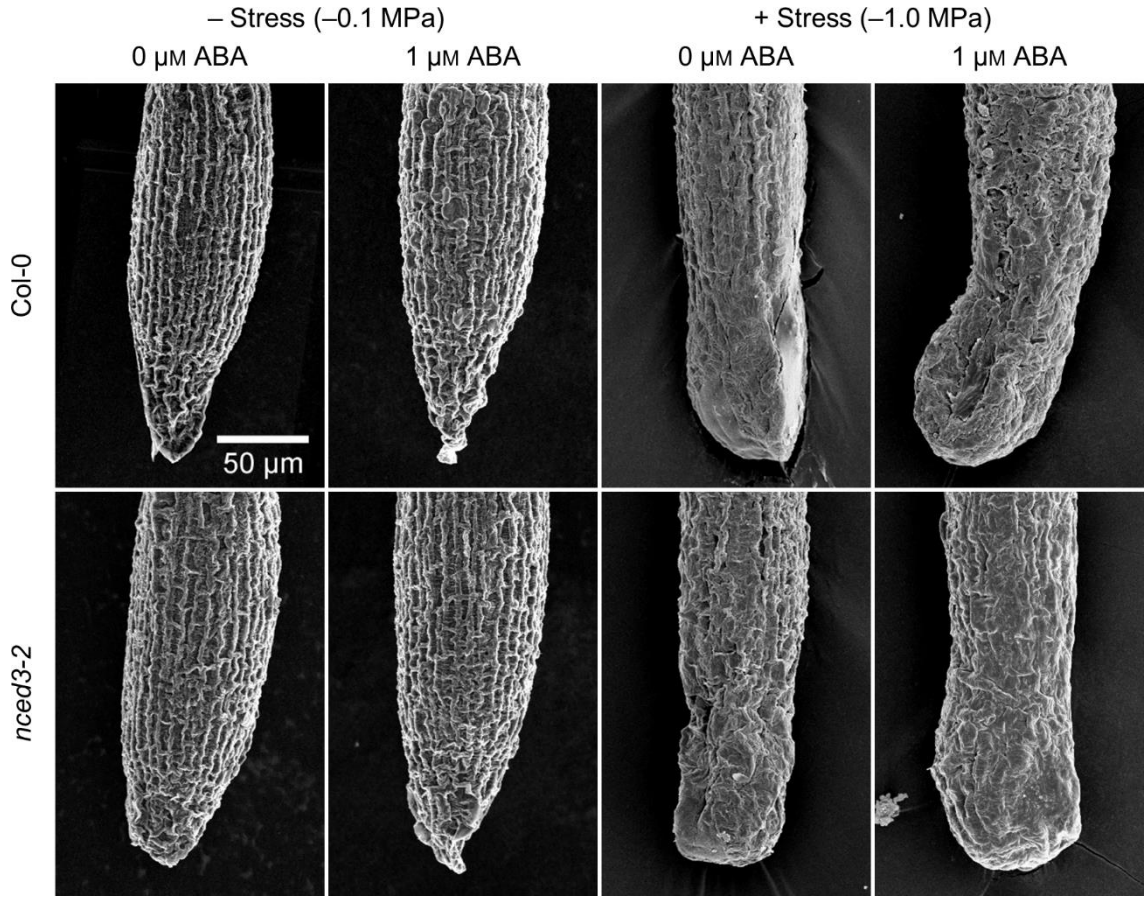

Fig. 3.10. Scanning electron micrographs of root tips of Arabidopsis wild-type Col-0 and *nced3-2* plants grown for 5 d in agar media at –0.1 or –1.0 MPa with 0 or 1 µM abscisic acid (ABA) (Study 8).

CHAPTER IV

CONCLUSIONS

The results in Chapter II demonstrate the effectiveness of ABA foliar spray in three different applications for vegetable transplants: stress control, height control, and extension of transplant marketability. The beneficial effects of ABA in these application strategies include stomatal closure and inhibition of stem elongation and leaf expansion. However, depending on species, cultivar, application rate, and growth stage, ABA foliar spray can also induce undesirable growth modifications, including reduced stem diameter, leaf chlorosis and abscission, and growth delay during field establishment. The effectiveness of ABA as a management tool is determined by the balance between the beneficial growth control and the undesirable growth modulation. Therefore, application rate and timing of ABA must be optimized based on the sensitivity of target crops.

The results in Chapter III suggest important morphological mechanisms of ABA-regulated growth modulation. Microscopic analysis of leaf epidermis revealed that ABA inhibits cell expansion, but not cell division or stomata formation, suggesting that the ABA-induced inhibition of leaf expansion is a mechanism to conserve water without limiting plant growth capacity. Leaf chlorosis induced by ABA occurs only in matured leaves and independently of ethylene synthesis. A proposed new mechanism is that ABA limits distribution of N into non-growing matured leaves, thereby inducing leaf-age dependent chlorosis. Furthermore, ABA exerts promotive effects on primary root elongation especially under water stress, despite dehydration-induced damage on root tip structures. These results suggest that the overall function of ABA in stress adaptation is to conserve water and nutrients to support new growth.

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